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A STUDY OF THE NITROGEN METABOLISM AND OF ACIDOSIS AFTER THE TRANSPLANTATION OF A URETER INTO THE DUODENUM IN DOGS.

By KINGO GOTO, M.D.

(*From the Department of Surgical Research and the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.*)

(Received for publication, November 19, 1917.)

Numerous reports, both clinical and experimental, have discussed the feasibility of implanting a ureter into some part of the intestinal canal, and have described methods of operative procedure. The literature was reviewed in 1909 by Steinke (1), who concluded that the results show that under certain conditions the ureter may be transplanted successfully. The failures, however, are numerous, the ultimate results uncertain, and a fatal termination is so apt to occur that no definite claim for the operation can as yet be made. Most experimental studies, as those recently made by Steinke (1909), Stewart (2) (1910), and Sweet and Stewart (3) (1914), have been especially concerned with the ascending infection of the kidney and the mechanical dilatation of the ureter following operation.

In order to avoid some of these difficulties—peritonitis, ascending infection of the kidneys, and stenosis of the ureteral orifice where it enters the intestine, with resulting hydroureter and hydronephrosis—Sweet and Stewart devised the following method of operation.

The right ureter of the dog is isolated and severed close to the bladder, and the free upper end passed through the lumen of the greater pancreatic duct into the intestine. This operation is made possible in the dog by the fact that this animal possesses at least two pancreatic ducts, both of which are separate from the bile duct. In Sweet and Stewart's series (3) eight dogs were used, and in some, nephrectomy of the left kidney was done at a later date. In none of the animals was there evidence of infection of the kidney, but in some, distinct evidence of obstruction was found, and in all, death resulted after varying intervals of time with symptoms suggestive of an intoxication.

In experiments such as those cited above an intoxication in the absence of infection suggests two possibilities: intoxication from (*a*) disturbance of kidney function or (*b*) absorption of urinary constituents draining into the intestine. It was with the hope of throwing

some light upon this intoxication that the present study was undertaken at the suggestion of Dr. Sweet.

EXPERIMENTAL.

Six dogs were used; in two a ureter-intestinal transplantation[1] was performed and four were used for various controls. The dogs were kept in metabolism cages and all were kept on the same dietary régime. The diet contained 0.4 gm. of nitrogen per kilo and 70 calories per kilo of body weight. It consisted of beef heart, lard, bread crumbs, sugar, a little salt (2 gm.), and sufficient bone ash to ensure well formed feces. The food was mixed with about 300 cc. of water. During each period of collection of blood and urine food was withheld. The animals were catheterized at the end of every 24 hours. After each catheterization the bladder was washed out and the wash water added to the catheterized urine and that which was voided naturally, and the whole made up to a definite volume.

The study of the urine included the determination of the total nitrogen, urea nitrogen, and ammonia nitrogen. For the determination of total nitrogen and urea nitrogen in the urine the new Nesslerization method of Folin and Denis (4) was used, and for ammonia the aeration method of Folin and Macallum (5). Blood was obtained by aseptic puncture of the vena jugularis and utilized for the study of the non-protein nitrogen, the urea nitrogen, and carbon dioxide content. In the examination of the blood the method of Folin and Denis (4) was used for the determination of non-protein nitrogen and urea nitrogen, and Van Slyke's method (6, 7) for the carbon dioxide content. Tests for ketones with Rothera's method (8) were made from time to time. All analyses were made in duplicate and sometimes in triplicate.

The operative procedure, as carried out by Dr. Sweet, consisted first, in transplantation of the right ureter into the duodenum and then, after several days, the removal of the left kidney. In all the experiments Period A refers to control observations before the first operation, Period B to observations after transplantation of ureter but before removal of the opposite kidney, and Period C to observa-

[1] All operations were done under ether anesthesia by Dr. J. E. Sweet.

tions after removal of the opposite kidney. During Period A animals were studied before any operative procedure for periods of 4 days. After the first operation several days were allowed for recovery from the acute effects of the operation, and another period of chemical study was undertaken (Period B). Under Period B1 are given the results obtained with animals after transplantation of the ureter, and under Period B2, as control, the results with animals in which the ureter was ligated and sectioned without removal of the kidney. After the opposite kidney was removed the chemical studies of the blood were continued until the dog died (Period C).

TABLE I.

Dog No.	Period.	No. of experiments.	Urine.			Blood.			Remarks.
			Total nitrogen per day.	Urea nitrogen per day.	Ammonia nitrogen per day.	Non-protein nitrogen per 100 cc. of blood.	Urea nitrogen per 100 cc. of blood.	Carbon dioxide content of plasma.	
			gm.	*gm.*	*gm.*	*mg.*	*mg.*	*vol. per cent*	
1, 2, 3, 4	A	4	4.12	3.15	0.15	29.5	10.1	56	Control observations in normal animals.
1, 5	B1	2	4.59	3.47	0.22	38.3	14.3	56	Right ureter in duodenum.
6	B2	1	6.18	4.44	0.18	30.3	11.1	52	" " ligated.
1, 5	C	2				201.8	102.2	41	Left kidney removed after Period B1.

It is obvious that in Period C the chemical examinations were necessarily limited to the blood.

In two other dogs as a control the right ureter was cut and ligated, but as one developed distemper following the operation the results on only one are given (Table I, Period B2).

The general results are shown in Table I which gives the average results for all animals during each of the three periods. In Tables II and III the results in Periods B and C are given in detail in contrast to the averages of Period A.

A comparison of Periods A and B1 shows that in the latter there is a moderate increase in the total nitrogen, urea nitrogen, and am-

TABLE II.

Dog 5.

Period.	Date.	No. of observations.	Weight.	Urine.			Blood.					Remarks.
				Total nitrogen per day.	Urea nitrogen per day.	Ammonia nitrogen per day.	Date.	No. of observations.	Non-protein nitrogen per 100 cc. of blood.	Urea nitrogen per 100 cc. of blood.	Carbon dioxide content of plasma.	
	1916		*kg.*	*gm.*	*gm.*	*gm.*	*1916*		*mg.*	*mg.*	*vol. per cent*	
A												
Average.....	Oct. 9–15	6	16.1	4.86	3.09	0.20	Oct. 13–19	5	33.4	11.2	57	Normal.
B	Oct. 24			6.93	4.47	0.35	Oct. 24		39.2	10.4	52	Oct. 19. Right ureter in duodenum.
	" 25		15.5	6.21	4.61	0.25						
	" 26	7	14.6	5.14	4.41	0.16	Oct. 26	4	42.8	18.2	48	
	" 27		14.5	6.43	4.29	0.29						
	" 28		14.3	5.48	4.61	0.41	Oct. 28		45.2	16.9	48	
	" 29		14.0	6.42	4.39	0.37						
	" 30		13.4	4.74	3.85	0.30	Oct. 30		50.0	23.4	59	
Average.....			14.4	5.91	4.38	0.31			44.3	17.2	51	Oct. 31. Left kidney removed.
C			13.8				Nov. 2		108.5	51.2	42	
			13.6				" 4		207.5	88.6	29	
			13.3				" 6	5	231.5	92.1	37	
			13.1				" 8		245.0	106.2	40	
			12.8				" 10		306.0	187.5	35	10 a.m. Died.

At autopsy slight hydronephrosis, cortex not greatly diminished; ureter not dilated but fluid could be forced into intestine only on pressure. Microscopically the kidney does not show evidence of infection.

monia of the urine and in the non-protein nitrogen and urea nitrogen of the blood. Whether this is due to retention of nitrogenous substances held back by injury to the kidney in consequence of the anastomosis, or to absorption of urine from the intestine, is a question which we shall discuss later. The change is relatively slight as compared with that of Period C in which a true nitrogen retention undoubtedly exists. In Dog 1 the probability of absorption of urinary constituents from the intestine cannot be entertained, for the amount of functioning kidney remaining was very slight and urine could be forced through the ureter only with great difficulty. Here, atrophy of the kidney and obstruction to the ureter, the opposite kidney having been removed, led to renal insufficiency and nitrogen retention. In Dog 5 the atrophy of the kidney was not so great, and ureteral obstruction doubtful; though this experiment is not so conclusive, it supports the view that the intoxication is due to retention and not to absorption from the intestine. The phenomena of Period C may be dismissed, therefore, as those of renal insufficiency and analogous to the changes observed by Karsner, Bunker, and Grabfield (9) after removal of both kidneys at consecutive operations. The changes in Period C in the carbon dioxide content of the plasma are noteworthy. A comparison with the findings in Period A, the control period, on an identical régime as regards food intake, indicates that the acidosis is due to change in kidney function. The search for ketones in these animals was negative. That all the changes we have found in the blood may arise after removing one kidney and ligating the opposite ureter is shown in Table IV.

The observations in Period B1 are difficult to explain. An increased output of urea and ammonia nitrogen in the urine and at the same time a retention in the blood cannot be connected with either disturbed elimination or absorption of urine from the intestine. Control observations on this point were unsatisfactory. In the animal in which, as control, one ureter was ligated without removal of the corresponding kidney (Period B2), the urinary output of nitrogenous bodies increased greatly but without appreciable change in those of the blood (Table I). Also, when in Period B2, of the experiment described here, urea is given by mouth, the nitrogen of both urine and blood is increased. These experiments, since they throw no

TABLE III.

Dog 1.

Period.	Date.	No. of observations.	Weight.	Urine.			Blood.					Remarks.
				Total nitrogen per day.	Urea nitrogen per day.	Ammonia nitrogen per day.	Date.	No. of observations.	Non-protein nitrogen per 100 cc. of blood.	Urea nitrogen per 100 cc. of blood.	Carbon dioxide content of plasma.	
	1916		*kg.*	*gm.*	*gm.*	*gm.*	*1916*		*mg.*	*mg.*	*vol. per cent*	
A Average.....	Nov. 13–16	4	15.2	3.63	2.99	0.15	Nov. 13–16	4	29.9	10.9	60	Normal.
B	Nov. 22	4	14.2	3.94	3.33	0.19	Nov. 22	4	32.6	18.8	59	Nov. 20. Right ureter in duodenum.
	" 23		13.9	5.36	3.94	0.19	" 23		32.5	15.2	62	
	" 24		13.9	5.27	3.78	0.18	" 24		33.4	15.2	61	
	" 25		13.7	4.84	3.36	0.16	" 25		34.2	14.7	64	
Average.....			13.9	4.85	3.60	0.18			33.2	16.0	61	
B (repeated).	Dec. 1	4	14.2	3.31	2.98	0.23	Dec. 1	4	29.6	9.9	58	
	" 2		13.9	3.29	2.27	0.22	" 2		30.2	10.4	56	
	" 3		13.7	2.78	1.26	0.17	" 3		34.1	8.0	58	
	" 4		13.5	2.73	2.15	0.15	" 4		35.2	10.3	56	
Average.....			13.8	3.03	2.16	0.19			32.3	9.9	57	

C			13.2				Dec. 9	3	81.2	60.8	50	Dec. 7. Left kidney removed.
			12.8				" 11		215.0	96.3	46	
			12.4				" 13		257.0	144.0	44	Died over night.

At autopsy marked hydronephrosis and hydroureter, only a thin rim of kidney cortex remaining. Fluid could be forced through the ureter into the intestine only on considerable pressure. Microscopically the kidney does not show evidence of infection.

light on the problem, are not reproduced in detail. Probably both after transplantation of the ureter (Period B1) and after its ligation and section without removal of the kidney (Period B2) there arises from some unknown cause an increased tissue catabolism accounting for the increased blood urea and non-protein blood nitrogen in Period B1 and for the increased nitrogenous excretion in the urine in Period B2. It is evident, however, that the explanation of the observations in Period B1 must await more detailed and more carefully controlled studies in which, among other things, the duodenal contents must be studied as to their content of nitrogen or other urinary constit-

TABLE IV.

Dog 6.

Date.	Blood.		
	Non-protein nitrogen per 100 cc. of blood.	Urea nitrogen per 100 cc. of blood.	Carbon dioxide content of plasma.
1916	*mg.*	*mg.*	*vol. per cent*
Nov. 18	34.8	10.4	52
" 19	29.3	9.4	53
" 20	28.2	10.2	55
" 21	26.6	8.3	52
Nov. 24	Left kidney removed; right ureter severed between ligatures; right kidney not removed.		
Nov. 25	53.4	17.1	45
" 26	79.6	32.8	43
" 27	122.0	44.6	44
" 28	154.0	105.0	44
" 29	198.0	113.0	43

uents, both in normal dogs and those with anastomosis, in order to determine whether urine enters the intestine in any amount. These observations have been made in the present study, but are not satisfactory on account of the great difficulties of control.

The marked accumulation of non-protein and urea nitrogen in the blood in Period C shows that the transplantation of the right ureter into the duodenum had so interfered with the function of the right kidney as to render it inadequate for maintaining renal function after removal of the left.

SUMMARY.

1. The present work was undertaken to study the metabolism in the dog after a ureter-intestinal transplantation. Four dogs, Nos. 1, 2, 5, and 7, were originally operated upon. Two, Nos. 2 and 7, showed kidney infection; the other two were not infected, and in these the metabolism was studied; one of the latter (No. 1) showed a marked hydronephrosis and hydroureter.

2. Both after the transplantation of the right ureter into the intestine and the ligation of the right ureter, there is generally a moderately increased output of nitrogen in the urine and, in the former instance especially, a retention of nitrogen in the blood, but no change in carbon dioxide content in the blood. The significance of this is probably an increased tissue catabolism, the cause of which is doubtful without further work.

3. After removal of the left kidney subsequent to transplantation of the right ureter into the duodenum, renal insufficiency and resulting retention developed. The non-protein and urea nitrogen in the blood steadily increased and the carbon dioxide content of the blood diminished to the level characteristic of a moderate acidosis. No ketones were found in the blood The dogs died 5 to 10 days after the nephrectomy under conditions characteristic of suspended renal activity—deep respiration, unconsciousness, and sopor.

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METHOD FOR INTRAVENOUS INJECTION OF GUINEA PIGS.

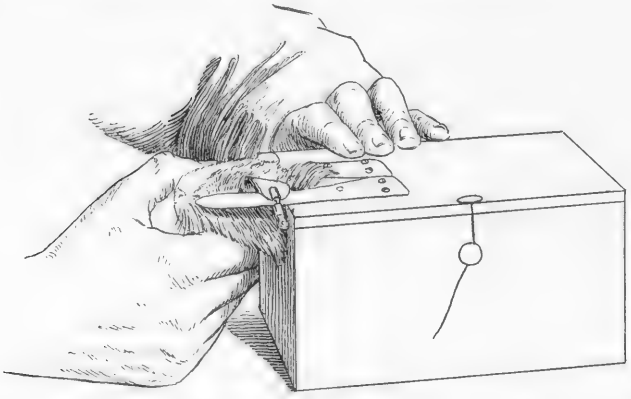
By PEYTON ROUS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

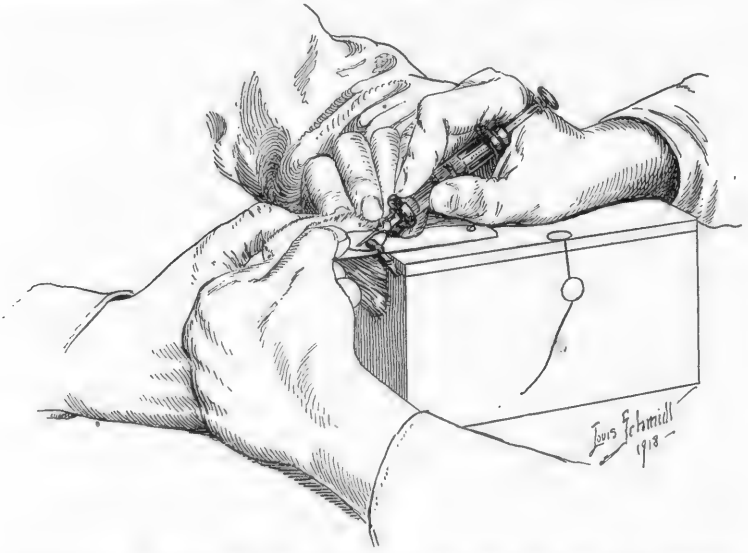
(Received for publication, January 25, 1918.)

A great drawback to guinea pigs as experimental material lies in the difficulty of intravascular injections. Direct cardiac puncture is, of course, readily performed, but much of an injected fluid may escape into the pericardial sac, or the pleural cavity, and the operator be none the wiser. Dosage is well controlled when the jugular vein is used, but this must be bared by incision, and day to day injections are well nigh impossible. To meet the requirements of such work a technique has been devised whereby repeated injections may be made into an ear vein.

Most guinea pigs have relatively large ear veins, and those near the margin are extemely superficial. They lie, indeed, so close to the surface that in the attempt to get into them with a needle the vessel is usually transfixed or so torn that hemorrhage obscures the field. A greater obstacle to the injections is encountered in immobilizing the animal. To accomplish this, the guinea pig is placed in a small wooden box with a U-shaped opening in one end through which the head is thrust (Text-fig. 1). Extra space in the box is packed with towelling, and the lid brought down and secured, with a flexible wire, as closely as the respiration will permit. Fixed firmly to the lid are two thin plates of bone which project horizontally above and to either side of the guinea pig's head. These are platforms to which the ears may be fixed during injection. When needle and syringe are ready, the head is pressed firmly up against the platforms by an assistant, and one of the ears is drawn over and fastened lightly with a rubber-covered spring clamp (Text-fig. 1). Then the injection is made, away from the clamp, into a marginal vein (Text-figs. 2 and 3).



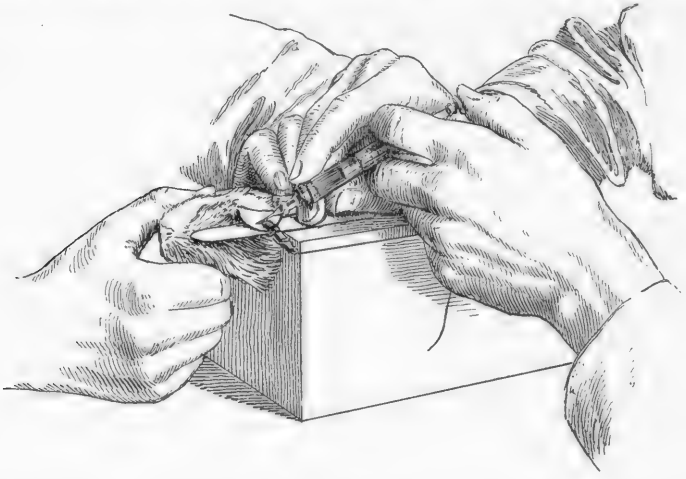
TEXT-FIG. 1. The animal's head is pressed firmly up against the platforms, to one of which the ear is fixed with a rubber-covered clamp.



TEXT-FIG. 2. With the thumb the ear is held flat and the vein distended. The bevelled side of the needle point lies against the vein as the latter is punctured.

In Text-figs. 2 and 3, the needle represented is of much larger caliber than that actually employed.

Some guinea pigs have small, indurated ears, the veins of which are not readily seen. These are unsuitable for use. Animals with black ears are a somewhat difficult material. In any instance a marginal vein should be chosen for the injection. The marginal vessels are not merely to be preferred: they are the only ones that can be used satisfactorily. The central veins, while larger, lie in a loose tissue which makes them difficult to pierce and renders leakage almost



TEXT-FIG. 3. The vein has merely been punctured, not entered further. Injection proceeds.

unavoidable. When the veins at the margin are used, any leak is at once evident: while the perivascular tissue is ordinarily so tough, that after a puncture either the injection proceeds successfully or no fluid leaves the syringe.

The needles employed should be fine (about No. 27 gauge, Brown and Sharpe), rather flexible, and at least half an inch long. Shorter ones are ordinarily so rigid as to be readily forced from the vein by any slight movement. It is important that the needle point should be bevelled obtusely. When an injection is given, the ear is held flat for the moment with the operator's thumb which by pressure distends the vein (Text-fig. 2). The needle is turned so that the

opening in it faces downward against the vessel as this is pierced. The merest puncture is sufficient. There should be no attempt to introduce the needle into the vein's lumen. All that is necessary is for an opening to be made in the vessel which will come opposite the opening in the needle. The tough perivascular tissue prevents any escape of material to either side. If the technique has been good, yet fluid fails to enter the circulation, the occurrence or absence of bleeding when the needle is withdrawn will show whether the vein has been punctured. If there is bleeding the needle may be thrust again into the little puncture wound, and now injection will often be successful. When large quantities of fluid are to be injected, the same puncture may be entered repeatedly.

The syringe should be absolutely tight and should contain no air, for the reason that successful puncture is most readily told by the slight yielding of the piston as the fluid begins to enter the vein; and this sign will be lacking when there is air in the barrel.

The technique has been described at length because strict adherence to it means success instead of failure. When it is rightly applied, injections up to 4 cc. are not difficult and may be accurately given day after day. The work is much less difficult than the injection of mice into the caudal vein, which is now so commonly practised.

A STUDY OF THE ANTISEPTIC PROPERTIES OF CERTAIN ORGANIC COMPOUNDS.*

By I. J. KLIGLER, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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Interest in the selective behavior of dyes dates back to Ehrlich's work with methylene blue. However, Stilling was probably the first to call attention to the antiseptic action of dyes on bacteria. He examined the inhibitive effect of diphenyl- and triphenylmethane dyes and recommended auramine and methyl violet as good antiseptic agents. Penzoldt tested a series of dyes and found that methyl violet, malachite green, etc., were good inhibitors. Von Drigalski and Conradi carried the idea a step further and showed that some aniline dyes inhibited *B. typhosus* less than other bacteria present in feces and recommended the use of crystal violet for the isolation of *B. typhosus* from stools, etc. Loeffler, and later Conradi, studied this phase of the problem more thoroughly, the latter testing about 400 dyes, but since they limited their observations to the action of these substances on *B. typhosus*, they contributed nothing new concerning the specific behavior of the dyes. Churchman, however, developed these observations and showed that certain violet dyes (gentian violet, crystal violet, dahlia) were more inhibitive for Gram-positive than for Gram-negative bacteria. These findings were confirmed and extended by Krumwiede and Pratt, who studied a larger series of this group of dyes.

It is evident from this brief review that the triphenylmethane dyes constitute a group of substances toxic to bacteria and reacting in a partially specific manner in the sense of Bechhold and Ehrlich. But, owing to the fact that the investigators were more absorbed in the practical application of this property, little is known concerning the nature of the action. Since my problem was similar to theirs, and since their extensive investigations resulted in only a partially successful solution, it seemed that a more effective attack might be made possible by a better understanding of the factors concerned in the specific affinity manifested by these dyes.

The noteworthy fact gathered from the literature is that all the dyes used in the isolation of *Bacillus typhosus* (crystal violet, malachite

* Work conducted under a grant from the International Health Board of The Rockefeller Foundation.

green, brilliant green), as well as those studied by Churchman (gentian violet, crystal violet, dahlia), belong to the triphenylmethane group. Diamino or triamino triphenylcarbinol may be considered the basis of these dyes. Upon treatment with acid, these dye bases are converted into dyes themselves, the conversion, as is commonly accepted, being accompanied by a rearrangement to the quinoid form. A series of homologous dyes may be produced by substituting the hydrogen in the NH_2 group by alkyl or aryl radicals. This class of substances promised, therefore, to be a good starting-point for the study of the structural chemical factors involved in the action of dyes on bacteria. A series of representative compounds was selected and their action on a number of typical bacteria studied quantitatively under carefully controlled conditions.[1] It soon became apparent that it would be desirable to extend the list and include for comparison a number of the simpler aromatic amino compounds. Consequently, aniline, toluidine, and some of their alkyl derivatives, and a few other related compounds were tested.[2]

Technique.

Substances Used.—A list of the substances used and their chemical constitution are given in Table I. The compounds are arranged as nearly as possible in the order of their complexity. The list is by no means exhaustive. Other compounds might have been included but were not easily obtainable, while still others were ruled out because of their insolubility.

Method.—The details of the method used in testing the antiseptic property of a given compound are highly important. Although comparable and fairly constant results may be obtained under identical conditions, a variation in one or another of the factors involved will cause decided fluctuations in the results. The important factors to be controlled are the composition and reaction of the medium and the condition of the culture used in the test.

[1] On account of the war, only a limited number of the compounds selected could be obtained.

[2] The dyes used were all Grübler's and were presumably fairly pure. The auramine, as well as the aniline, toluidine, and other compounds tested, was kindly furnished by Dr. W. A. Jacobs of The Rockefeller Institute for Medical Research, and were obtained from either Kahlbaum or Schuchardt.

TABLE I.

List of Compounds Used.

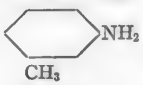
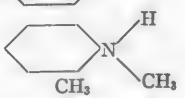
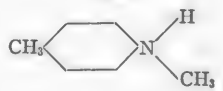
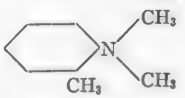
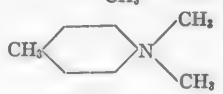
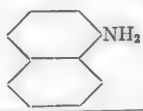
No.	Name.	Formula.
1	Methylamine (mono-).	$NH_2.CH_3.HCl$
2	" (di-).	$NH(CH_3)_2.HCl$
3	" (tri-).	$N(CH_3)_3.HCl$
4	Methyl alcohol.	$CH_3.OH$
5	Ethyl "	$CH_3.CH_2.OH$
6	Ethylamine.	$NH_2.C_2H_5$
7	Diethylamine.	$NH(C_2H_5)_2$
8	Aniline.	$C_6H_5.NH_2$
9	Methyl aniline.	$C_6H_5.NH.CH_3$
10	Dimethyl "	$C_6H_5.N(CH_3)_2$
11	Ethyl "	$C_6H_5.NH.C_2H_5$
12	Diethyl "	$C_6H_5.N(C_2H_5)_2$
13	*o*-Toluidine.	$C_6H_4.CH_3.NH_2$
14	*m*- "	$C_6H_4.CH_3.NH_2$ 
15	*p*- "	$C_6H_4.CH_3.NH_2$
16	N-Methyl *o*-toluidine.	$C_6H_4.CH_3.NH.CH_3$ 
17	N- " *p*- "	$C_6H_4.CH_3.NH.CH_3$ 
18	N-Dimethyl *o*- "	$C_6H_4.CH_3.N(CH_3)_2$ 
19	N- " *p*- "	$C_6H_4.CH_3.N(CH_3)_2$ 
20	α-Naphthylamine.	$C_{10}H_6.NH_2$ 

TABLE I—*Continued.*

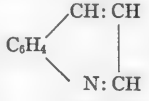
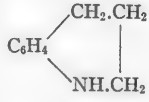
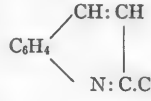
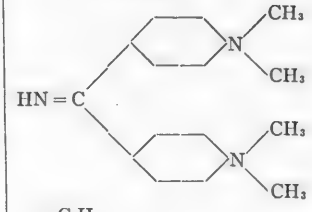
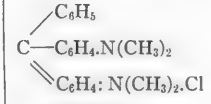
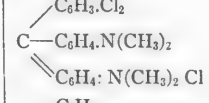
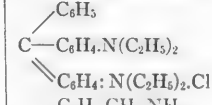
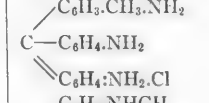
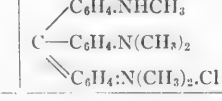
No.	Name.	Formula.
21	Quinoline.	
22	Tetrahydroquinoline.	
23	Quinaldine.	
24	Auramine.	
25	Malachite green.	
26	Victoria "	
27	Brilliant "	
28	Fuchsin (acid).	
29	Methylviolett B.	

TABLE I—*Concluded.*

No.	Name.	Formula.
30	Crystal violet.	$C(C_6H_4.N(CH_3)_2)_2(C_6H_4:N(CH_3)_2.Cl)$
31	Gentian "	Mixture of crystal and methyl violet plus dextrin.
32	Methyl green.	$C(C_6H_4.N(CH_3)_2.CH_3Cl)(C_6H_4.N(CH_3)_2)(C_6H_4:N(CH_3)_2.Cl)$
33	Aniline blue.	$C(C_6H_3.CH_3.NH.C_6H_4)(C_6H_4.NH.C_6H_5)(C_6H_4:NH.C_6H_5.Cl)$
34	Dahlia.	$C(C_6H_3.CH_3.NH.C_2H_5)(C_6H_4.NH.C_2H_5)(C_6H_4:NH.C_2H_5.Cl)$

Composition of the Medium.—That the presence of different amounts of colloidal or organic matter influences the action of dyes is well known. This is equally true of other antiseptics. An idea of the effect of different media on the antiseptic action of chemicals is obtained from the data shown in Table II. In these tests the only variable was the composition of the test media; a special peptone broth and standard beef extract broth were used. It is clear from the results that although the characteristic action of the drug is not modified by the medium, the effective concentration is appreciably altered. It is important, therefore, in testing a large number of drugs, to use a medium subject to as little variation in composition as possible. The following medium proved satisfactory:[3]

	per cent
Peptone	1.0
Potassium phosphate (dibasic)	0.5
Sodium chloride	0.5
Glucose	0.1

[3] Fairchild's peptone was used throughout the investigation. The salts and sugar were chemically pure.

TABLE II.

Action of Antiseptics in Peptone and Nutrient Broth Respectively.

Test substance.	Dilution.	Test media.	Test cultures.*				
			2	6	17	12	14
Dibromo-β-naphthol.	12,500	Nutrient broth.	−†	−	−	+++	+++
	25,000	" "	+	−	++	+++	+++
		Peptone "	−	−	−	+++	+++
	40,000	" "	+	+	++	+++	+++
Hexamethylenetetramine quaternary salt of *p*-chloroacetylaminotetraethyl *p'*, *p''*- diaminotriphenylmethane.	15,000	Nutrient broth.	+	+	+	++	+++
		Peptone "	−	−	−	+	+
	30,000	Nutrient "	+	+	+	+++	+++
		Peptone "	−	−	−	+	++
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	10,000	Nutrient broth.	−	−	−	+	−
	20,000	" "	+	±	±	+++	++
		Peptone "	−	−	±	++	−
	40,000	" "	+	+	+	+++	++

* The numbers of the cultures correspond with those given in Table IV.

† − indicates no growth; ±, +, ++, +++ indicate the relative amount of turbidity at the end of 24 hours.

This culture fluid varies little, if at all, is easily prepared, and serves as a favorable substratum for all the cultures used in these tests.

Reaction of the Medium.—The reaction of the test medium in terms of hydrogen ion concentration is of even greater importance. This fact has been entirely overlooked until recently (Wright, 1917). A few preliminary tests showed that not only was the antiseptic power affected, but the specific behavior towards different organisms was also modified. Typical results are given in Table III. The medium given above eliminated this factor, because it had a constant pH of 7.1, the reaction usually favorable for growth of bacteria; also, since it required no adjustment, it was not subject to variation on that account.

TABLE III.

Effect of the Reaction of the Test Medium on the Action of Antiseptics.

Test substance.	Dilution.	Reaction of test medium.*	Test cultures.†				
			2	6	17	12	14
		pH					
		6.2	±	±	±	+	±
Caffeine.	1: 100	7.4	±	±	±	±	−
		8.2	±	∓	±	±	−
		6.2	−	−	−	+++	±
Hexamethylenetetramine quaternary salt of *p*-chloroacetylamino-leukomalachite green.	1: 10,000	7.4	+	+	±	+++	++
		8.2	+	+	+	+++	++
		6.2	−	−	−	++	++
Dibromo-*β*-naphthol.	1: 12,500	7.4	−	−	−	+++	++
		8.2	−	−	+	+++	+++
		6.2	−	−	−	−	−
Hexamethylenetetramine quaternary salt of chloroacetylethylamine.	1: 10,000	7.4	−	−	−	+	−
		8.2	−	−	−	++	±

* Nutrient broth was used in all these tests. A quantity of broth was prepared and tubed and N acid or alkali added sterilely to give the desired pH.

† The cultures and symbols are the same as in Table II.

Test Cultures.—The condition of the test culture was the third important factor that had to be considered. Variations are likely to occur either because of lack of adaptation to the test fluid or on account of the inherent fluctuations of the organism itself. To eliminate the former, the cultures were grown in the test broth for at least 3 days, daily subcultures being made; the latter were partly controlled by using wherever possible recently isolated organisms and more than one strain of each type. The strains used, together with their origin and some descriptive remarks, are given in Table IV.

Procedure.—The procedure given below was followed throughout the work. The peptone broth was put up in flasks and autoclaved. 5 cc. portions were then pipetted out sterilely into sterile test-tubes and incubated in a saturated incubator over night. Solutions of the substance to be tested were made up in sterile water and graded amounts added to the broth to give the desired concentration. The stock so-

TABLE IV.

Culture No.	Name.	Source.	Remarks.
123	*B. aerogenes.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; typical.
14	*B.* "	Isolated from stool, 1916.	Indol +; Voges-Proskauer reaction −; not typical; behaves like *B. aerogenes.*
24	*B. cloacæ.*	American Museum of Natural History.	Indol −; Voges-Proskauer reaction +; does not liquefy.
11	*B. coli (communis).*	Isolated from stool, 1916.	Indol +; sucrose −.
13	*B.* " "	" " " 1916.	" +; " −.
12	*B.* " *(communior).*	" " " 1916.	" +; " +.
15	*B.* " "	" " " 1916.	" +; " +.
17	*B. typhosus.*	" " Patient O., 1916.	Agglutinated with typhoid serum. Culturally typical.
19	*B.* "	Isolated from Carrier L., 1916.	
20	*B.* "	Isolated from Carrier C., 1916.	
2	*B. dysenteriæ* Flexner.	Old Institute laboratory stock.	Maltose − (Hiss-Russell).
24	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose + (Flexner).
26	*B.* " "	Isolated by Dr. Smillie, 1916.	Maltose − (Hiss-Russell).
6	*B.* " Shiga.	Institute stock strain (Gay).	Reacts typically.
27	*B.* " "	Isolated by Dr. Smillie, summer, 1916.	" "
30	*B.* " "	Albany stock 114 F.	" "
21	*B. proteus.*	Isolated from stool, 1916.	
106	*B. subtilis.*	American Museum of Natural History.	
347	*Staphylococcus aureus.*	American Museum of Natural History.	

lutions were made up of such strength that no more than 1 cc. or less than 0.1 cc. had to be added to give the proper dilution. The cultures to be inoculated were filtered through sterile cotton filters, diluted with broth to give uniform turbidity, and a large standard loop was inoculated into the broth tubes. The tubes were then incu-

bated for 24 hours and the growth was recorded in terms of degree of turbidity. This gave the inhibitive power of the substance in 24 hours. In a few cases the killing power in 2 and 24 hours respectively was ascertained by subculturing the broth tubes to agar slants. This procedure was not carried out systematically because of the time consumed and since for the purposes of the study it was sufficient to determine the inhibitive property in a constant time limit.

RESULTS.

The results are given in Table V. The compounds used are arranged in the order of their increasing antiseptic power, and the results recorded in terms of the highest inhibiting dilution (first row) and the dilution which just failed to inhibit (second row). These two figures indicate the limits of the zone in which the inhibiting dilution lies. Whenever the difference between the two figures was too great, additional tests were made to reduce the gap. When more than one test was made, the average of the results was taken, and when more than one strain of a given organism was used, the average for the type is given. On the whole, individual strains of the typhoid, dysentery, or *aerogenes* bacilli varied but little, while more decided fluctuations were obtained with *Bacillus coli*. The repeated tests checked fairly closely with the original ones.

The facts brought out in Table V are difficult to summarize. In general, it is clear that on starting with aniline or its mono- or dimethyl derivatives, the introduction of a methyl group in the nucleus, as seen in the behavior of the corresponding toluidine derivatives, results in a definite increase in the inhibitive power of the compound. This is also evident from the contrast between quinoline and quinaldine. Similarly, the antiseptic property is enhanced by the substitution of either methyl or ethyl radicals in place of the hydrogens in the NH_2 group. The amount of increase, up to a certain point at least, depends on the number and character of the alkyl radicals introduced. A second alkyl produces a more marked rise than the first, while an ethyl group is more effective than a methyl radical. This general phenomenon is also observed among the dyes. Beginning with fuchsin there is a progressively increasing antiseptic action which

TABLE V.

Inhibition of Growth of Bacteria by Certain Chemical Compounds.

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A.	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Methyl alcohol.	10*	10	5	10	10	10			
	20	20	10	20	20	20			
Ethyl alcohol.	15	15	10	20	25	25			
	25	25	20	25	40	40			
Aniline.	300	250	250	350	450	450	350	500	1,000
	350	300	300	400			400	1,000	5,000
o-Toluidine.	475	475	475	475	600	600			
	550	550	550	550	1,000	1,000			
p-Toluidine.	475	475	450	550	600	600			
	550	550	475	600	650	650			
Methyl aniline.	650	600	600	650	950	950	650	1,000	6,000
	700	650	650	700	1,000	1,000	700	5,000	10,000
Ethyl aniline.	1,000	1,000	1,000	1,100	1,350	1,350	1,100		
	1,100	1,100	1,100	1,200	1,500	1,500	1,200		
Methyl *o*-toluidine.	1,100	950	950	1,100	1,400	1,400	1,100		
	1,350	1,100	1,100	1,350	1,600	1,600	1,350		
Methyl *p*-toluidine.	1,100	950	950	1,100	1,400	1,400	1,100		
	1,350	1,100	1,100	1,350	1,600	1,600	1,350		

Dimethyl aniline.	1,350 1,500	1,350 1,500	1,350 1,500	1,500 1,750	1,750 2,000	1,750 2,000	1,100 1,350		
Dimethyl *o*-toluidine.	1,550 1,750	1,550 1,750	1,550 1,750	1,750 2,100	2,100 2,500	2,100 2,500	1,350 1,550		
Dimethyl *p*-toluidine.	2,500 2,650	2,650 2,900	2,500 2,650	2,900 3,200	3,500 5,000	3,500 5,000	2,500 2,650		
Diethyl aniline.	4,500 5,400	4,500 5,400	4,500 5,400	6,000 7,000	7,000 8,500	7,000 8,500	4,000 4,500		
Quinoline.	1,400 1,600	1,100 1,400	1,100 1,400	1,600 2,100	1,600 2,100	1,600 2,100	1,100 1,400		
Tetrahydroquinoline.	1,600 2,100	1,400 1,600	1,400 1,600	1,600 2,100	1,600 2,100	2,100 5,000	1,400 1,600		
Quinaldine.	2,600 3,400	2,100 2,600	2,100 2,600	2,600 3,400	2,600 3,400	3,400 5,000	2,100 2,600		
α-Naphthylamine.	2,600 3,100	2,600 3,100	2,100 2,600	2,600 3,100	3,100 7,500	3,100 7,500			
Auramine.	1,800 2,200	2,200 2,700	2,200 2,700	2,700 3,500	2,700 3,500	4,200 5,200	1,500 1,800	6,000 10,000	10,000 25,000
Fuchsin.	 8,000	8,000 12,000	12,000 15,000	12,000 15,000	100,000 110,000	110,000 200,000	12,000 150,000	500,000 1,000,000	300,000 500,000
Malachite green.	 20,000	40,000 45,000	45,000 50,000	30,000 35,000	250,000 500,000	500,000 1,000,000	35,000 40,000	4,000,000 5,000,000	1,000,000 2,000,000

* The numbers indicate dilutions; the first row the inhibiting dilution, the second the one which failed to inhibit.

TABLE V.—*Concluded.*

Antiseptic compound.	Gram-negative.							Gram-positive.	
	B. aerogenes.	*B. coli* A.	*B. coli* B.	*B. typhosus.*	*B. dysenteriæ* F.	*B. dysenteriæ* S.	*B. proteus.*	*B. subtilis.*	*S. aureus.*
Victoria green.		60,000	70,000	40,000	700,000	700,000	40,000	5,000,000	1,000,000
	40,000	70,000	80,000	50,000	1,400,000	1,400,000	50,000	10,000,000	2,000,000
Dahlia.		55,000	65,000		110,000	110,000	55,000	2,000,000	800,000
	45,000	65,000	75,000	45,000	150,000	150,000	65,000	4,000,000	1,000,000
Methylviolett B.		70,000	75,000	60,000	175,000	175,000	85,000	4,000,000	800,000
	60,000	75,000	85,000	65,000	250,000	250,000	95,000	5,000,000	1,000,000
Gentian violet.	25,000	85,000	90,000	50,000	100,000	100,000			
	50,000	95,000	100,000	60,000	200,000	200,000			
Crystal violet.		85,000	100,000	85,000	400,000	400,000	200,000	4,000,000	1,000,000
	60,000	95,000	110,000	95,000	800,000	800,000	250,000	5,000,000	2,000,000
Brilliant green.	100,000	675,000	550,000	510,000	1,500,000	2,000,000	675,000	15,000,000	4,000,000
	175,000	800,000	600,000	550,000	2,000,000	3,000,000	800,000	20,000,000	5,000,000
Methyl green.	1,200	1,200	1,200	1,200	8,000	8,000	2,700	50,000	30,000
	2,200	2,200	2,200	2,200	15,000	15,000	3,500	100,000	50,000
Aniline blue.	1,200	1,200	1,200	1,200	1,200	1,200	1,200		

runs parallel with the increase in the number of methyl or ethyl groups. The triethyl derivative is about as effective as the hexamethyl, while the tetraethyl is the most active of the series. The behavior of the *o*- and *p*-dimethyl toluidines indicates that position may be a factor in determining the degree of antiseptic action. The effect of the introduction of chlorine into the benzene nucleus is seen from the differences between malachite green and victoria green. This effect on the introduction of halogen has been observed before in other classes of compounds. It is also interesting to note that the substances containing two aromatic nuclei, namely naphthylamine, quinoline, quinaldine, and the diphenylmethane dye, auramine, are more potent than the corresponding monophenyl derivatives, whereas the triphenyl derivatives are the most active of the substances tested.

It would seem, then, that the inhibiting effect of these substances is due on the one hand to aniline with the benzene nucleus as its basis, and on the other to the number of these nuclei. The effect is consistently enhanced by the addition of alkyl radicals, either to the nucleus, or to the amino group. The number and character of these radicals also determine the degree of effectiveness.

An exception to the general phenomenon of the increase of inhibitive action produced by the increase in the number of alkyl groups is seen in the anomalous behavior of methyl green. This substance is identical with crystal violet, with the exception that one of the tertiary nitrogens of this dye has been changed as a quaternary salt by the addition of methyl chloride. Contrary to the expectation of an increase in antiseptic power, this dye is almost inert. It is also noteworthy that in the case of the triphenyl derivative of rosaniline, aniline blue, in which the hydrogens are substituted by phenyl groups, there is a decided reduction in inhibitive action.

In some respects, these results are in accord with those obtained by other workers with other classes of compounds. The well known difference between phenol and cresol and the observations of Jacobs, Heidelberger, and Bull of the progressively increasing bactericidal action, on proceeding from the dimethyl to the diethyl and dipropyl derivatives of certain quaternary salts of hexamethylenetetramine, may be cited as instances.

While it is possible to point to the factors concerned in the enhancement of the germicidal power, it is difficult to account for the specific behavior of these substances. All the compounds tested are decidedly more active against the Gram-positive than the Gram-negative bacteria. This is true as well of aniline and its methyl derivative as of auramine and the triphenylmethane dyes. It is interesting to note that while aniline and its derivatives are more active against *Staphylococcus aureus* than *Bacillus subtilis*, the converse is true of the dyes.

Partial specificity becomes most marked in the triphenylmethane dyes. This is particularly evident in their more potent action against the Gram-positive organisms and the dysentery bacilli. Though the dysentery bacilli are exceedingly sensitive to the action of these substances, they are decidedly less so than the Gram-positive bacteria. These dyes are also markedly inactive against *Bacillus aerogenes* and, with the exception of fuchsin, are more inhibitive for *Bacillus coli* than for *Bacillus typhosus*.

The fact that all these dyes behave alike, irrespective of the number and character of the alkyl radicals, indicates that the molecule as a whole is concerned with the partial specific action. This is in accord with the fact that three of these dyes have been used in the isolation of *Bacillus typhosus*.

Mention should be made of the side-light which the behavior of these organisms towards this group of substances throws on their possible relationship. *Bacillus typhosus* and *Bacillus aerogenes* (also *Bacillus paratyphosus* B) are more sensitive to the simple aniline derivatives and less so to the dyes than *Bacillus coli*. The latter, as well as *Bacillus dysenteriæ*, is relatively much more sensitive to the dyes than to the aniline compounds. The extreme sensitiveness of the dysentery bacilli to the action of the dyes is especially interesting. While there was little difference between the behavior of the two classes, the Flexner cultures invariably showed a greater tolerance for fuchsin than did the Shiga bacillus. Their extreme sensitiveness to this class of chemical compounds renders it unlikely that any representative of the group may be found that will be of service in isolating them from polluted materials.

CONCLUSIONS.

This study of the inhibitive effect of aniline and some of its derivatives and of the triphenylmethane dyes on certain bacteria warrants the following tentative conclusions:

1. The composition and reaction of the medium exert a marked influence on the behavior of the antiseptic. The higher the concentration of organic nitrogenous compounds (peptone) in the medium, the lower is the effective concentration of the dye. The reaction of the medium modifies the specific action of the antiseptic, owing probably to an alteration in the bacterial cell.

2. The germicidal action of the compounds is a function of the benzene nucleus, the added elements or radicals, their number, and, in the case of the dyes, probably the quinoid structure of the nucleus.

3. As far as tested, the increase in the number of alkyl radicals increases the antiseptic power. Methyl green is an interesting exception to this rule, for the change of one of the nitrogens to the quaternary salt is accompanied by an almost complete loss in inhibitive action.

4. The antiseptic power is enhanced to a greater extent by an ethyl than a methyl group, and the second alkyl produces a proportionately greater increase than the first. It appears that the relative position of the introduced group may be a factor in determining the relative improvement in the effectiveness of the compound.

5. The introduction of a methyl group in the nucleus consistently enhances the inhibitive action of the compound and its alkyl derivatives. This is evident from a comparison of the action of aniline and its derivatives with that of toluidine and its corresponding derivatives.

6. The simple aniline derivatives, as well as the dyes, are more toxic for the Gram-positive than the Gram-negative bacteria. Of the former, *Bacillus subtilis* is more sensitive to the dyes than *Staphylococcus aureus*, while the reverse is true in the case of the aniline compounds.

7. The most marked specific selective effect is manifested by the triphenylmethane dyes. *Bacillus acrogenes* and *Bacillus typhosus* possess a higher resistance to these substances than *Bacillus coli* or

Bacillus dysenteriæ. The last is exceedingly sensitive. This partial specificity is apparently a function of the molecule as a whole.

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THE ELIMINATION OF IRON AND ITS DISTRIBUTION IN THE LIVER AND SPLEEN IN EXPERIMENTAL ANEMIA. II.

By HARRY DUBIN, Ph.D., and RICHARD M. PEARCE, M.D.

(*From the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.*)

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In an earlier communication (1) we presented the results of a study of the elimination and storage of iron in transient experimental anemias due to a single injury. These we found were not characterized by an increased elimination of iron in the feces, or in the urine, except as an accompaniment of hemoglobinuria. On the other hand, an increased storage of iron in the liver and spleen was constantly observed. These observations support well known views concerning the power of the body to conserve iron, and suggest that some other factor besides blood destruction must be operative in those hemolytic anemias in man characterized by excessive elimination of iron in the feces. The natural assumption is that in the hemolytic anemias there exists some disturbance of the mechanism concerned in the retention or conservation of iron, and we have now studied a chronic type of experimental anemia in order to determine whether chronicity may be a factor. In the present series of experiments *Trypanosoma equiperdum* has been used to cause a continuous blood destruction in dogs. It was hoped that in the long continued and progressive anemia so produced we might find an experimental condition approximating the hemolytic anemias of man.[1]

EXPERIMENTAL.

As our methods of experimentation and analysis have been described in a previous paper (1), they need not again be given. The

[1] Only the study of iron metabolism is presented here. For a general description of these experiments see Krumbhaar, E. B., Experimental trypanosomiasis: *T. equiperdum* infection in the dog, *J. Infect. Dis.*, in press.

TABLE I.

Iron Elimination in Urine and Feces in Trypanosome Anemia with and without Splenectomy.

Dog No.	Date of infection.	Blood picture.			Period.	Iron.			Remarks.
		Date.	Hemoglobin.	Red blood corpuscles.		Urine per day.	Feces per day.		
			per cent			*mg.*	*mg.*	*per cent*	
1	Nov. 1 and 17	Nov. 2	94	6,550,000	Nov. 1–7	1.39	20.7	0.138	No splenectomy.
		" 7	72	5,220,000					
		" 28	42	3,550,000	Nov. 28–Dec. 4	1.48	18.6	0.118	
		Dec. 4	48	4,980,000					
2	Dec. 15	Nov. 11	99	6,880,000	Nov. 14–20	1.68	23.7	0.123	No splenectomy. Dec. 19. Fragment of spleen removed.
		Jan. 8	61	3,660,000	Jan. 9–15	1.59	24.7	0.128	
3	Jan. 11	Jan. 13	115	7,800,000					No splenectomy.
		" 27	84	4,720,000	Jan. 23–27		18.6	0.103	
4	Jan. 8	Jan. 13	111	7,400,000					No splenectomy.
		" 27	93	5,900,000	Jan. 23–27		15.8	0.109	
5	Nov. 1	Nov. 2	99	7,280,000	Nov. 1– 7	1.43	18.7	0.131	
		" 6	82	6,240,000					
		" 28	45	3,600,000	Nov. 28–Dec. 4	1.63	18.0	0.105	
		Dec. 4	40	3,680,000					Dec. 13. Splenectomy.
		" 18	44	3,510,000	Dec. 18–22	6.08*	12.8*	0.099	
6	Jan. 23	Dec. 9	94	6,520,000	Dec. 9–15		24.7	0.114	Feb. 10. Splenectomy.
		Feb. 13	30	2,900,000	Feb. 12–16		20.4	0.119	
7	Control.	Nov. 11	105	7,100,000	Nov. 14–20	1.20	15.4	0.127	Nov. 27. Splenectomy.
		Dec. 4	104	7,480,000	" 28–Dec. 4	1.39	16.8	0.112	
8	Control.	Jan. 10	107	7,440,000	Jan. 9–15		29.6	0.100	Jan. 22. Splenectomy.
		Feb. 14	104	7,050,000	Feb. 10–16		30.1	0.111	
9	Control.	Jan. 10	92	6,900,000	Jan. 9–15		19.7	0.120	Jan. 22. Splenectomy.
		Feb. 14	88	6,410,000	Feb. 10–16		20.1	0.117	

* Feces mixed with urine.

results of the study of the elimination of iron in the feces are presented in Table I, in which are given also some figures for the urine. The urine was not studied in all experiments inasmuch as our earlier work demonstrated that in the absence of hemoglobinuria the iron content of urine does not vary. Six of the animals were infected with trypanosomes and two of these were splenectomized.[2] In addition the table presents three control experiments on non-infected animals with studies of iron elimination before and after splenectomy.[3] The figures are in accord with those obtained in our earlier study (1) of transient anemia and prove conclusively that an experimental anemia with continuous blood destruction is not necessarily characterized by an increased elimination of iron in the feces. In this connection we consider Experiments 5 and 6, in which splenectomy was done at the stage of severest anemia, very important. The removal of the spleen of the normal dog, does not, as has been shown in other work from this laboratory (2), lead to an increased elimination of iron, but no observations are at hand concerning iron elimination after removal of the spleen in an anemic dog. Experiments 5 and 6 offer the needed evidence to show that iron elimination after splenectomy, in the anemic as in the normal dog, remains unchanged.

In Table II may be seen the figures showing the distribution of iron in the spleen and liver. The normal percentages for spleen and liver, as we have shown elsewhere (1), are respectively 0.185 and 0.104. The figures for trypanosome anemia are sometimes higher, but in general are in accord with those obtained in the study (1) of transient anemia. Experiments 1, 2, 3, 4, 10, and 11 are simple studies of trypanosome anemia. In Experiments 12, 13, and 14 arsenobenzol,[4] a trypanocide, was given to delay the fatal termination in the hope of obtaining a greater accumulation of iron in the liver and spleen. These animals thus suffered a severe initial anemia, an improvement as the trypanosomes disappeared under treatment, and eventually,

[2] These operations were done under complete ether anesthesia.

[3] The averages of all figures for iron elimination in the earlier study are as follows: urine, 1.6 mg. per day; feces, 18.9 mg. per day, or 0.116 per cent of dry weight.

[4] The arsenobenzol (Schamberg) was furnished by the Dermatological Research Laboratories of the Polyclinic Hospital, Philadelphia.

TABLE II.

Iron in Liver and Spleen in Experimental Trypanosome Anemia.

Dog No.	Date of infection.	Degree of anemia.*			Iron in the dry organ.		Remarks.
		Date.	Hemoglobin.	Red blood corpuscles.	Spleen.	Liver.	
			per cent		*per cent*	*per cent*	
1	Nov. 1 and 17	Dec. 7	48	4,980,000	0.270		Dec. 19. Fragment of spleen removed.
2	Dec. 15	Jan. 16	56	3,900,000	0.285	0.101	Jan. 18. Autopsy.
3	Jan. 11	Feb. 6	72	3,700,000	0.573	0.186	Feb. 9. "
4	" 8	" 6	50	4,570,000	0.478	0.176	" 6. "
10	Feb. 6	" 28	68	4,700,000	0.632	0.217	Mar. 2. "
11	Mar. 22	Mar. 31	90	3,130,000	0.466	0.133	Apr. 5. "
12	Feb. 6†	May 1	40	4,000,000	0.293	0.224	May 6. " Feb. 29, Mar. 1 and 2. Salvarsan.
13	Apr. 22†	June 5	55	3,690,000	0.431	0.235	June 6. " May 16–24. Salvarsan.
14	" 22†	May 29	54	3,540,000	0.315	0.186	" 6. " " 16–22. "
5	Nov. 1	Dec. 18	44	3,510,000	0.274‡	0.104	Dec. 13. Splenectomy. Dec. 22. Autopsy.
6	Jan. 23	Feb. 13	30	2,900,000	0.422‡		Feb. 10. " Feb. 17. "
7	Dec. 15	Nov. 27	112	7,240,000	0.179§	0.178	Nov. 28. " Dec. 22. Died. Autopsy.
8	Mar. 28	Jan. 24	110	7,700,000	0.180§		Jan. 22. " May 11. Autopsy.
		May 1	35	3,940,000		0.290	
9	Apr. 2	Jan. 20	96	7,200,000	0.174§	0.117	" 22. " Apr. 5. "
15	Mar. 9	Mar. 26	45	3,920,000	0.455‡	0.159	Mar. 26. " Mar. 29. "
16	Apr. 22	May 26	23	2,610,000	0.605‡	0.171	May 22. " May 27. Died.
17	Feb. 2†	Mar. 3	72	5,090,000	0.353‡		Mar. 2. " Apr. 7. Autopsy. Mar. 9. Salvar-
		Apr. 3	20	2,590,000		0.264	san.
18	Apr. 21	May 5	60	5,150,000	0.181§	0.181	Apr. 13. Splenectomy. May 4. Autopsy.
19	" 21	" 1	90	7,880,000	0.190§	0.135	" 13. " " 7. "

* The degree of anemia represents blood examination most nearly approximating in point of time the removal of the organ.

† In this animal treatment by salvarsan caused a disappearance of trypanosomes and improvement of the anemia but with eventual return of parasites in the circulating blood and recurrence of anemia.

‡ Splenectomy followed infection.

§ Splenectomy preceded infection.

with the reappearance of the parasites in the circulating blood, a recurrence of anemia, terminating fatally. Despite the long course of the experiment the storage of iron was not greatly augmented in either liver or spleen. The results, in general, are in accord with the work of Boycott and Price-Jones (3), who, in a study of experimental trypanosome anemia in the rabbit, found the iron of the liver to be double that of normal animals while the iron of the spleen was increased twenty times.

In Experiments 7, 8, 9, 18, and 19 the spleen[2] was removed before, and in Experiments 5, 15, 16, and 17 after infection to see whether the absence of the spleen led to greater storage of iron in the liver. No evidence of this is at hand.

TABLE III.

Iron Elimination in Bile.

Dog No.	Period.	Iron.			Remarks.
		Urine-bile mixture per day.	Feces per day.		
		mg.	*mg.*	*per cent*	
20	Nov. 1– 7	1.84	30.4	0.109	Nov. 1. Bile duct-ureter anastomosis.
	" 11–17	1.97	36.4	0.123	
21	Nov. 1– 7	1.75	7.3	0.119	Nov. 1. Bile duct-ureter anastomosis.
	" 11–17	1.91	6.7	0.118	
	" 28–Dec. 4	1.51	10.0	0.127	Nov. 24. Splenectomy.

In seven animals with anemia the iron content of the kidney was determined, the figures varying from 0.044 to 0.067 per cent of dry substance, essentially normal figures.

In another series of experiments, the elimination of iron in the bile was studied, in order to determine whether a loss occurred through this channel. For the collection of bile we used the method of Pearce and Eisenbrey (4), which consists in an anastomosis, after removal of one kidney, of the corresponding ureter with the bile duct.[2] By this procedure the bile passes into the urinary bladder and is available, without loss, for the quantitative chemical examination of any constituent not present in the urine. As we had at hand many control

figures for iron in the urine, there was no possibility of confusion as to figures for iron in the urine and bile respectively. In Table III are shown the results in two experiments. The figures for urine and bile combined approach so closely those for urine alone that this line of study was abandoned with the conclusion that in the dog iron in appreciable amounts is not eliminated in the bile.

However, in the animals with bile duct-ureter anastomosis an increased storage of iron was found in the spleen. As anemia was not

TABLE IV.

Iron Storage in the Spleen after Exclusion of Bile from the Intestine.

Dog No.	Blood picture. Date.	Red blood corpuscles.	Hemoglobin.	Iron in spleen.	Remarks.
			per cent	*per cent*	
20	Nov. 7	6,820,000	103		Nov. 1. Bile duct-ureter anastomosis.
	Dec. 14	7,600,000	105	0.498	Dec. 19. Splenectomy.
21	Nov. 7	7,100,000	102		Nov. 1. Bile duct-ureter anastomosis.
	" 17	6,680,000	108	0.548	" 24. Splenectomy.
22	Feb. 7	6,020,000	86		Jan. 23. Bile duct-ureter anastomosis.
	Apr. 14	5,820,000	92	0.253	Apr. 17. Splenectomy.
23	Mar. 27	6,860,000	92		Feb. 6. Bile duct-ureter anastomosis.
	May 22	3,830,000	35	0.472	Anemia due to toluylenediamine.
					May 24. Autopsy.
24	Apr. 14	6,610,000	110		Jan. 24. Bile duct-ureter anastomosis.
	May 15	4,690,000	45		Anemia due to toluylenediamine.
	" 19	5,210,000	75	0.598	May 24. Autopsy.

produced artificially and did not develop spontaneously in three animals of the series this increased storage of iron is difficult to explain. It is possible that the loss of bile from the intestine may have been a factor, but as a satisfactory explanation is not at hand, the results are given in Table IV without further comment.

SUMMARY.

In the continuous blood destruction, essentially a chronic experimental anemia, caused by infecting the dog with *Trypanosoma equi-*

perdum, no increased elimination of iron is observed in the feces. The storage of iron in the liver and spleen under these experimental conditions is somewhat greater in amount, but of the same general character as in transient experimental anemia.

Splenectomy before or after infection, *i.e.* the development of anemia, influences neither the elimination of iron in the feces nor its storage in the liver.

The retardation of the course of the trypanosome infection and thus the production of a more chronic anemia by treatment with a trypanocide, arsenobenzol, likewise does not affect iron storage.

These experiments have therefore failed to reproduce the changes in iron metabolism seen in certain of the chronic hemolytic anemias of man.

In the presence of a bile duct-ureter fistula the iron content of the mixture of urine and bile is not appreciably greater than that of the urine alone. In the dog, therefore, the elimination of iron in the the bile would not appear to be an important factor.

On the other hand, when bile is excluded from the intestine an unusual storage of iron occurs in the spleen. For this no explanation is offered.

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A STUDY OF THE BLOOD PRESSURE BY THE METHOD OF GAERTNER, ESPECIALLY IN PATIENTS SUFFERING FROM FIBRILLATION OF THE AURICLES.

By ALFRED E. COHN, M.D., and CHRISTEN LUNDSGAARD, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

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INTRODUCTION.

A number of years ago Mackenzie (1) called attention to the difficulty in estimating the blood pressure in persons suffering from fibrillation of the auricles. There was general agreement that the difficulty existed, but there seemed to be no method for improving the technique of making clinical determinations. The beats which were heard first by auscultation or felt first on palpation, when pressure in the cuff system was allowed to escape, continued to be taken as the measure of the systolic pressure. A method was then suggested by James and Hart (2), later called the fractional method by Kilgore (3), by which not the maximum pressure of a few beats is taken as the measure of pressure, but the average of the systolic pressures of all the beats. Their technique consists in counting for 1 minute the number of beats felt below the brachial cuff at the radial artery at levels 10 mm. of mercury apart. The figure obtained is multiplied by the level of pressure, all the products are then added, and the sum is divided by the apex rate. Slight modifications and certain extensions of the method have since been suggested by Kilgore. He employed auscultation in addition to palpation, especially in calculating diastolic pressure.

This method presents certain difficulties, but before considering them we wish to review again certain points underlying the current clinical technique of estimating blood pressure. The peripheral arteries are regarded as representing an elastic reservoir kept filled by the action of the heart. It is the tendency of this reservoir to return to a position of rest, and in doing so to empty itself continuously into the smallest arteries and capillaries. By this effort these vessels are kept constantly filled.[1] So far as the constancy of the filling is concerned it makes no difference whether the elastic reservoir is filled by

[1] Cases in which there is a capillary pulse are exceptions to this rule.

volumes of blood equal in amount as normally, or unequal as in fibrillation of the auricles; or whether these volumes are delivered at equal or at somewhat unequal intervals. It matters only that the total volume in succeeding units of time, minutes for instance, is kept constant. Under ordinary circumstances this maintenance of constant volume is accomplished by uniform filling, but, failing this, it can be brought about by a compensating increase (or decrease) of resistance offered at the outflow of the reservoir system. It is an advantage to have the minute volume output of the left ventricle divided in roughly equal fractions throughout the minute. The point at which the reservoir empties into the smaller vessels may be regarded as the point where the head of pressure in the reservoir becomes effective. We speak then of the "effective pressure"[2] at this point.

The clinical habit of estimating arterial pressure in the brachial artery is adopted, not because the pressure here is especially important, but because it serves, on the one hand, as a rough indicator for the pressure level at the root of the aorta or in the left ventricle itself; that is, a difference is assumed between aortic and brachial pressure, sufficiently constant to permit the brachial pressure to serve as a valuable guide. On the other hand, brachial pressure may also serve as a suitable guide for judging the level of effective pressure, the pressure at the exit from the arterial into the capillary system. So far as the capillary system is concerned, there is no difficulty in this convention as long as the filling of the reservoir is maintained by even and uniform strokes; when the strokes are no longer uniform as in fibrillation, the difference between effective pressure, which tends to stay constant or alters only within narrow limits, and brachial pressure, which varies, must fluctuate so that the brachial pressure can no longer serve as an index of effective pressure.[3]

These considerations are important in relation to the technique of estimating systolic pressure when the auricles are fibrillating, for it is precisely in this condition that brachial pressure fluctuates and fails as an index of effective pressure. It has been found on closer study (3) that in auricular fibrillation not only the systolic, but also

[2] This phrase is used by James and Hart (2).

[3] See experiments reported by Cohn, A. E., and Lundsgaard, C., *J. Exp. Med.*, 1918, xxvii, 505.

the diastolic pressure of succeeding beats varies. These fluctuations necessarily result in irregularities in the pulse pressure. The pulse pressure does, in fact, undergo large alterations in the brachial artery. Further toward the periphery, however, the pulse pressure becomes progressively smaller, until, in the capillaries, it ceases to exist[1] and the flow is constant and continuous. The point at which this takes place is the point where, as has been said, the head of pressure becomes effective. Here a constant reading can be obtained. But since for clinical purposes no practicable technique exists for this, a point just proximal to the capillaries may be chosen. In the small arteries, of the size of the digital, the pulse pressure is small and alterations from mean pressure can from beat to beat be shown to be relatively unimportant.[4] For taking the pressure at this point a technique already exists. It is the method formerly employed by Gaertner and described by him in 1899 (4). The values obtained may be regarded as effective blood pressure. Readings so obtained are direct and the technique is simple. By this method we have taken the blood pressure of a few persons suffering from fibrillation of the auricles and have plotted, parallel to these curves, others made day after day by the fractional method of James and Hart, with the view of ascertaining the difference between the brachial and digital pressures. We have, in addition, studied a number of other individuals, some with normal and some with abnormal hearts, but all having hearts the mechanism of which was normal.

Technique.

The following technique was employed. One finger,—the ring finger,—was rendered bloodless by rolling a thick rubber ring from the tip to a small pneumatic cuff which was applied to the first (base) phalanx. The cuff corresponded exactly in plan to that of von Recklinghausen except that it was 2.5 cm. wide and about 5 cm. long. The cuff was connected by pressure tubing to a mercury manometer as in the von Recklinghausen plan. The pressure in this system was raised with a pump after the finger was blanched by the rubber ring. The ring was then removed and the pressure in the manometer cuff system

[4] This point is described in our report, *J. Exp. Med.*, 1918, xxvii, 505.

allowed to fall gradually and regularly.[5] When the pressure in this system falls to the level of the blood pressure, blood begins to flow into the finger distal to the cuff and is recognized by the return of color in the finger. After a little practice there is no difficulty in recognizing the return of color. It is important to make the estimations with north light. We have been aided in seeing the return of color, even in the very anemic, by laying the finger and hand to be examined on a dark, blue-gray cloth, to provide a proper contrast. It was the custom to read the pressure in each case 10 times and to average the readings. Of 153 determinations the range was 1 to 5 mm. in 11 determinations; 6 to 8 mm. in 69; 9 to 10 mm. in 35; 11 to 15 mm. in 27; 16 to 30 mm. in 7; 21 to 25 mm. in 2; and 26 to 30 mm. in 2. That is, the range was below 10 mm. in 75 per cent of the determinations. In many instances both observers made independent readings. When that was done, we compared the averages in twenty-six instances and found a difference of 2 to 4 mm. Twice the averages differed by 5 mm.; twice by 6 mm.; once by 7 mm.; twice by 8 mm.; once by 9 mm.; and once by 11 mm. The differences in range are greater than were found in the estimation of brachial pressure by Kilgore and his associates (5) but not so great as to render the method unserviceable. As an example of the method followed, we cite the figures of one patient, Case 2334 (Tables I and II), made by two observers.

Studies of blood pressure by Gaertner's method have been made with the view of comparing the readings found at the digital, with those taken at the brachial artery.

Hayashi (6), in Strümpell's clinic, found a difference of about 20 mm. between the readings taken of these two arteries whether they were made in children, men, or women, in infections, or in cardiac, vascular, or renal diseases. There were fluctuations in his figures but these were not of sufficient importance to alter the conclusion that the fall in pressure from the brachial to the digital vessels was about 20 mm. Doleschal (7) compared the pressure in the radial artery

[5] We provided for the gradual and regular fall in pressure by placing the rubber bulb which served as a pump between the jaws of a wooden vice. The distance between the jaws was regulated by a screw turned by a long shank; the use of a long shank permits more uniform motion. As the screw was released, the jaws of the vice were separated by springs properly placed.

TABLE I.

March 26, 1917.

Tonometer readings.

No. 1.	No. 2.
mm. Hg	*mm. Hg*
93	93
89	95
92	95
88	95
87	93
91	93
94	92
95	93
94	95
93	92
Average.....91.6 = 92^{+3}_{-4}	93.6 = 94^{+1}_{-2}

TABLE II.

Blood pressure by the fractional method.

Auscultation.		Palpation.	
Pressure.	No. of beats heard	No. of beats felt.	Pressure.
mm. Hg			*mm. Hg*
140	0	0	
130	31	20	130 × 20 = 2,600
120	25	22	120 × 2 = 240
110	9	26	110 × 4 = 440
100	9	61	100 × 35 = 3,500
			6,780
90	21	59	
80	18	61	
70	7	58	
60	2	60	$\frac{6780}{62}$ = 109
50	0	56	
Average pressure.....130		109	
Radial rate..... 62			
Apical " 62			

taken by means of von Basch's sphygmomanometer with that taken by Gaertner's tonometer and found uniformly that the tonometer readings were lower than those taken with von Basch's instrument. These results were naturally to be expected, and show that even where the pressure differences are probably small, as in the case of the radial and digital arteries, the tonometer readings, as we anticipate, are lower. Of 200 cases, for instance, the readings showed differences up to 5 mm. in 89, to 10 mm. in 80, to 15 mm. in 19, to 20 mm. in 6, and from 20 to 40 mm. in 6.

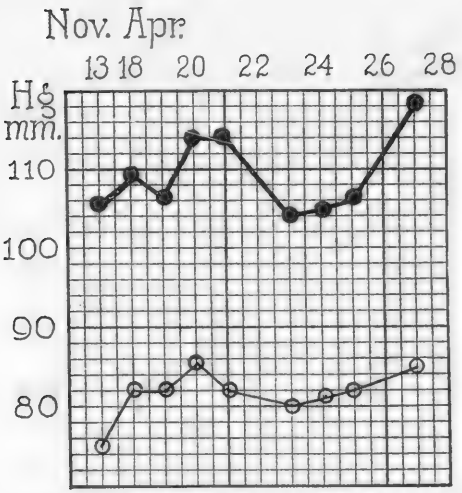
Our experience corresponds closely with that of Hayashi and Doleschal, for we have found uniformly that the tonometer readings at the digital arteries are always lower than the brachial readings. In eight normal individuals we found the average difference to be 20 (19.8) mm., the maximum being 30 mm., the minimum 5 mm. (Table III).

TABLE III.

No. of individual.	Brachial systolic pressure by auscultation.	Tonometer.	Difference.
	mm. Hg	*mm. Hg*	*mm. Hg*
1	110	96	14
2	120	92	28
3	116	97	19
4	119	98	21
5	110	96	14
6	114	109	5
7	120	90	30
8	109	81	28
Average.....			19.8

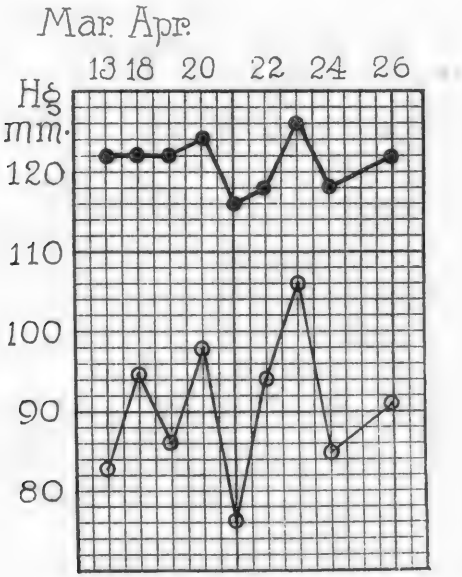
In the patients suffering from chronic heart disease and chronic nephritis (Table IV), in whom the mechanism of the heart beat was normal, the average difference between the two was 25 mm. in all cases, the range being from 6 to 80. If two cases (Nos. 10 and 12), suffering from complete heart block, are omitted, the average is 18 and the range 6 to 30. These values do not differ from normal.

If the cases are arranged according to the height of the brachial pressure, no correspondence between this and digital pressure is observed. The reason probably depends on the instability known to exist in the vasomotor mechanism in different individuals. It is pre-



——— Brachial pressure estimated by the method of auscultation.
— — — Brachial pressure estimated by palpation.
——— Digital pressure.

TEXT-FIG. 1. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.



TEXT-FIG. 2. Curves of the brachial and digital pressure of an individual in whom the circulation was normal.

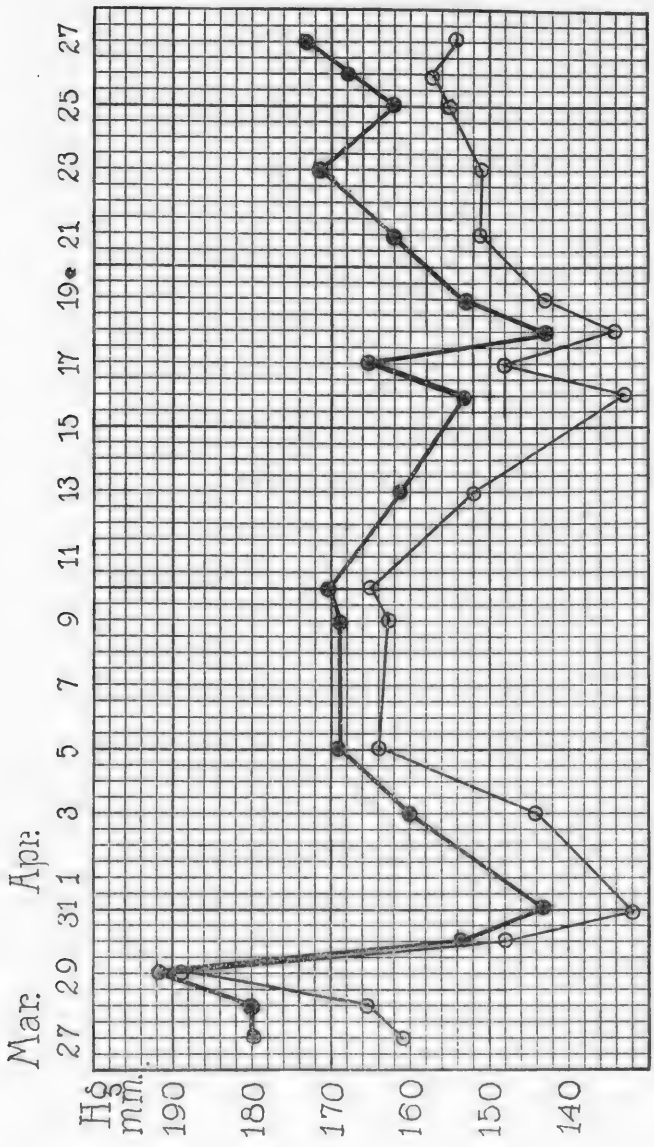
cisely in the smaller vessels that lability is observed, so that fluctuation is expected in the digital rather than in the brachial artery. It is for this reason that the readings in the two arteries are not parallel. In some respects the violent fluctuations in digital pressure present difficulties, so that as a guide for comparison pressure in the digital artery is unsatisfactory. The question arises, however, whether the difficulty which is found is not of clinical importance, and whether it ought not to be emphasized rather than evaded entirely as is now done in estimating pressure only at a point such as the brachial ar-

TABLE IV.

Case No.	Hospital No.	Rate.	Brachial systolic pressure.	Tonometer.	Difference.
			mm. Hg	*mm. Hg*	*mm. Hg*
1	587	80	84	65	19
2	2915	86	100	84	16
3	2069	109	103	73	30
4	2681	96	145	126	19
5	2867	83	134	106	28
6	2862	82	145	128	17
7	2961	90	153	147	6
8	2907	82	160	139	21
9	2336	85	176	169	7
10	1266	30	176	135	41
11	2992	92	180	162	18
12	2833	30	250	170	80
Average.....					25.1

tery where the least variation is found. We recall that the method of Gaertner was practically abandoned because of the fluctuations inherent not so much in the technique as in the artery itself. Furthermore, the matter is important on account of questions associated with fluctuations in oxygen unsaturation in the venous blood of the arm such as Lundsgaard (8) has found. These phenomena may find their explanation, in part, in facts like those which are shown here.

Curves of the brachial and digital pressure of two individuals in both of whom the circulation was normal illustrate these points. In the first instance (Text-fig. 1) the pressure in the brachial artery fluctuated within the usual narrow limits from 104 to 118 mm., a range of



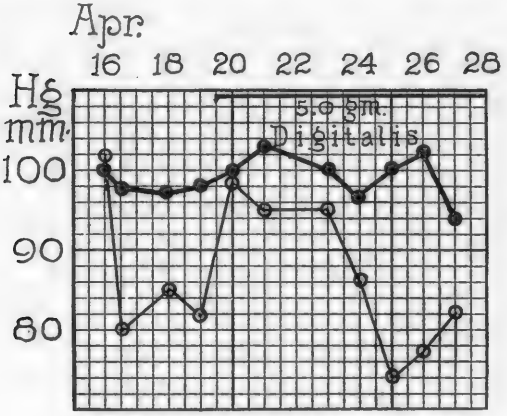
TEXT-FIG. 3. Curves of the brachial and digital pressure of a patient suffering from chronic nephritis and heart failure.

14 mm. Parallel digital pressure fluctuated between 75 and 85 mm., a range of 10 mm. The digital pressure was more constant than the brachial. In the second instance (Text-fig. 2) the brachial pressure fluctuated between 116 and 126 mm., a range of 10 mm., while the digital pressure fluctuated between 76 and 106 mm., a range of 30 mm., or three times as great as the brachial pressure. We expected the peripheral circulation of the first individual to be stable, but in the second we anticipated, because of the frequent rapid alterations of vasomotor tone seen in phenomena like dermatographia, that it was labile. Studies of blood flow, or oxygen unsaturation, already mentioned, had indicated that phenomena of the nature we are now emphasizing, existed to explain the obscure facts relating to flow. There is reason, therefore, to think that in studies like this, facts of importance may be found.

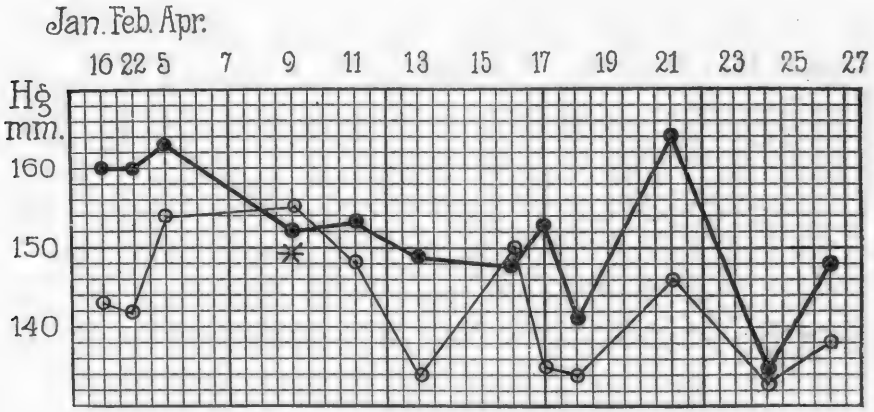
In this connection a consideration of the records of a patient (Text-fig. 3) are important. This individual suffered from chronic nephritis and heart failure; he was at first edematous and orthopneic and had a high pressure and an alternating pulse. The digital pressure fluctuated between 132 and 189 mm., a range of 57 mm. But the brachial systolic pressure fluctuated almost as violently between 143 and 192 mm., a range of 49 mm. In this instance the fluctuations are almost parallel and indicate that they depend on alterations in the functional state of the heart rather than on fluctuations in vasomotor tone. These three cases show that there are instances in which simultaneous brachial and digital readings are of value. But we have cited them in addition to show that of the two arteries, the blood pressure is uniformly higher in the one more centrally placed; the curves of the two do not cross.

OBSERVATIONS.

Our especial interest was directed to the study of the relation of digital to brachial pressures in individuals suffering from fibrillation of the auricles. We studied four patients in detail. The pressure curves made by the tonometer method are not remarkably different from those found when the mechanism of the heart beat is normal. But when the digital pressure curves are compared with the brachial pressures obtained by the method of fractional readings devised by



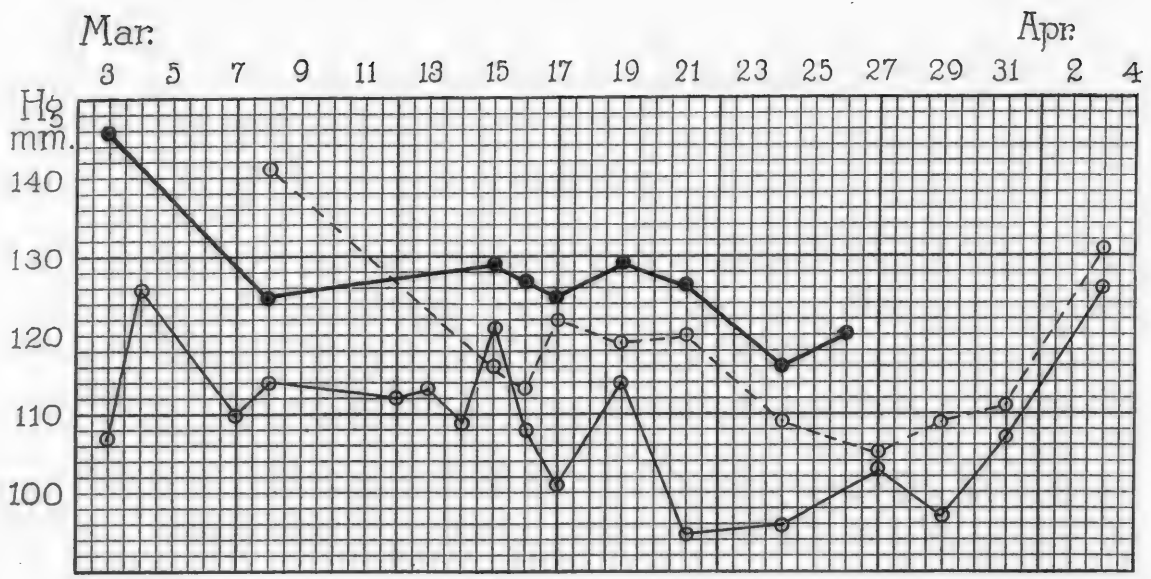
TEXT-FIG. 4. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once.



TEXT-FIG. 5. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross twice. The upper curve represents pressure in the brachial artery determined by the method of auscultation, except where indicated by an asterisk. The lower curve represents digital pressure taken by Gaertner's method.

James and Hart, difficulties occur. In the first place, the taking of readings requires as many minutes or half minutes as there are levels at which pressure is read. During this time the state of the vessels does not remain the same, the number of sounds heard or beats felt at one level may, after 3, 4, or 5 minutes, alter appreciably; sometimes, indeed, no reliable count is possible. To avoid continuous pressure, we invariably reduced the pressure to zero, for at least 1 minute between successive readings, although the length of the examination was thereby doubled. The procedure, however, did not remove the difficulty. The explanation for this may depend on a number of causes related to the auscultatory method, possibly to the formation of the sounds. But another cause also deserves consideration. Compression of the brachial vessels for so long a time may alter the partition of the blood stream so that an increasing fraction passes, uncompressed by the cuff, in the medullary vessels of the bone or in vessels protected by the grooves on its surface. This explanation implies a rapid increase in the area of the collateral vessels, but there is reason for believing that it may take place. It has, on the other hand, been observed that the difficulty may develop gradually. If absent at first, it increases as the daily examinations proceed. The establishment of the collateral circulation need, therefore, not be abrupt. The factors involved in the production of the sounds, whether produced by the mechanism of the water-hammer or otherwise, may also change. Whether these are the occurrences which actually take place and are the ones responsible for the difficulty we are considering, is of secondary importance. The sounds in any event do not remain sufficiently distinct in many individuals to make auscultation reliable as the basis of the fractional method. For the reasons stated, counting the beats by palpating the radial artery also offers difficulties. We have, therefore, in two cases counted the rate in the radial artery by palpation as well as in the brachial by auscultation. In one case in which it was satisfactory, we used the auscultatory method alone.

A second difficulty is that the curve of average brachial pressure occasionally crosses and is lower than the level of digital pressure taken on the same day. A crossing of the two curves had not been observed by others and was not seen in our studies of individuals the



TEXT-FIG. 6. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross once when the fractional pressure by palpation is considered.

mechanism of whose hearts was normal. The crossing of the curves is, moreover, inconceivable. In Case 13 (Table V) crossing was observed once in eleven observations (Text-fig. 4); in Case 14 (Text-fig. 5) twice in twelve observations; in Case 15 (Text-fig. 6) once in seventeen observations when the fractional pressure was taken by palpation, but not if taken by auscultation; in Case 16 (Text-fig. 7) thirteen times (in two they were equal) in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation.

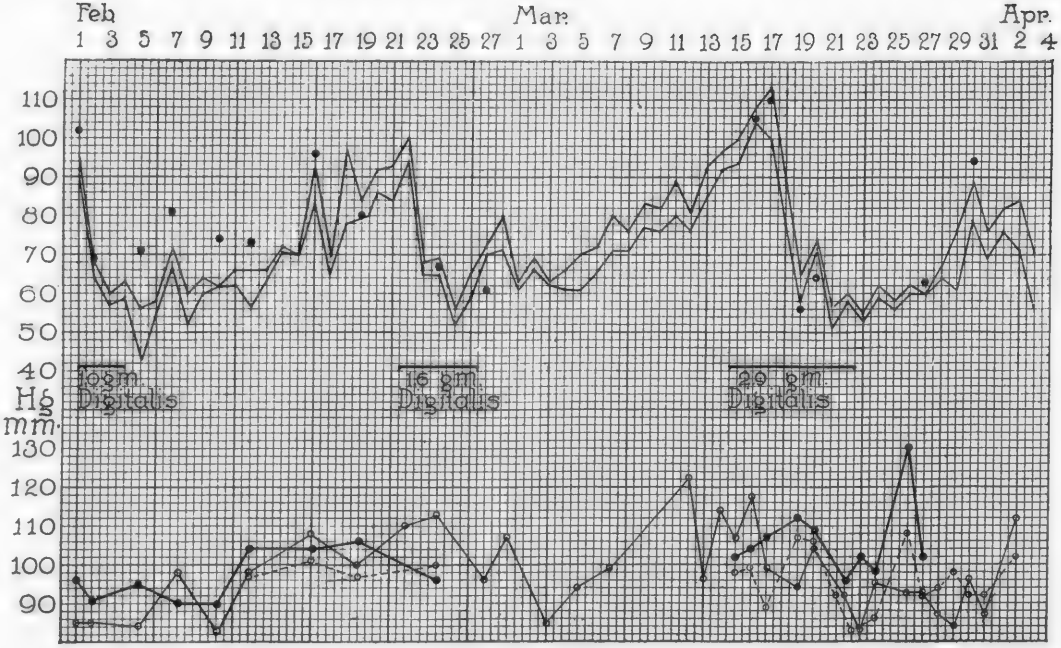
TABLE V.

Case No.	Hospital No.	No. of observations.	Crossing.	
			Observed by method of palpation.	Observed by method of auscultation.
13	3007	11		1
14	2548	12	1	1
15	2683	17	1	
16	2334	19	13	
		20		5

Although the number of patients studied is not large, the fact that in each of them the fractional method of estimating pressure gave readings for the brachial artery lower than for the digital is important. The finding makes it desirable to review the opportunities for error.

An average pressure can no doubt be obtained by the fractional method. But even if the result is obtained free from the technical errors already discussed, and from one other now to be considered, it would probably be incorrect because it is not possible to regard the circulation as is usual in physical phenomena. In order to do this the value of one cycle ought to be directly comparable with another in terms of its numerical equivalent.[6] To regard it in this manner is, however, impossible. If, for instance, a ventricular cycle is unable to lift the aortic valves, this cycle is of no importance in increasing or even in maintaining the pressure in the arterial reservoir. It has no functional value and ought not to be included in calculating the average pressure; it tends merely to lower the value. The only

[6] Kilgore has considered this matter in a similar manner.



TEXT-FIG. 7. Curves of the brachial and digital pressure of an individual in whom the auricles were fibrillating. The curves cross thirteen times in nineteen observations if taken by palpation, and five times in twenty observations if taken by auscultation. In the upper set of curves the upper line represents the average ventricular rate for the day, the lower one, the average radial rate. The dots indicate the ventricular rate at the time of blood pressure determination. In the lower set of curves the brachial pressure by the auscultatory method is represented by the heavy line; brachial pressure by the method of palpation is represented by the broken line. Digital pressure taken by the method of Gaertner is represented by the light line.

ventricular cycles which can have an influence on the pressure in the arteries are those the systolic value of which is greater than diastolic pressure. But as far as maintaining the driving force of the circulation is concerned, that is the head of pressure represented by the difference between systolic and diastolic pressure, not all the cycles having a systolic value above diastolic pressure are equal. Many having a low value need have no significance in maintaining the effective pressure. In cases where the rate is low, and where there is no pulse deficit, whether the rhythm is regular or irregular, both a high systolic and a high diastolic level can be maintained. This is observed in cases of complete heart block. Of two such cases studied, the brachial systolic pressure in one was 250, the diastolic 90, and the digital pressure 170; and in the other the systolic pressure was 176, the diastolic 63, and the digital pressure 164. It is apparent that additional ventricular beats, if they are ineffective, whether able to lift the aortic valves or not, would have no significant influence on these figures. The rate in both cases was about 30. A few cycles of great power can therefore maintain a high head of pressure. We infer from this that 30 to 40 beats at a given pressure, 130 mm. for instance, are sufficient to maintain effective pressure close to this level. If additional 40 to 60 beats having low systolic pressure values are introduced into the calculation the numerical value given to the pressure is merely reduced and no added light is thrown on the actual circulatory condition. And if ventricular cycles that do not even develop force sufficient to open the aortic valves are included, the average pressure is still further depressed.

In estimating the systolic pressure in fibrillation of the auricles, therefore, the fractional method is defective. A substitute is readily found in the use of the tonometer method of Gaertner. By its means satisfactory readings are possible. One should remember, however, that the readings so obtained tend to be about 20 mm. lower than the pressure in the brachial artery. As a rough method for estimating the pressure in fibrillation, it appears to us to be sufficient to find rapidly the level of pressure in the brachial artery at which about 40 beats per minute occur; this point is not far removed from the effective pressure about which information is actually required. It avoids, furthermore, the appearance of numerical accuracy, where accuracy, as Mackenzie pointed out, is impossible.

In two patients (Nos. 13 and 16), the subjects of fibrillation of the auricles, we were able to study the effect of digitalis on the brachial and digital pressures. In the first, after giving digitalis for about 8 days, there was a slight lowering of the digital curve (Text-fig. 4) from 97 to 74 mm. But in the period before this, readings of 80 to 85 mm. were recorded and a reading of 84 was found later in the treatment. The influence of digitalis in this instance cannot, therefore, be called striking. In the second case (Text-fig. 7) there were three digitalis periods separated by 14 or more days during which 1, 1.6, and 2.9 gm. were given. In each digitalis period the digital pressure fell, rising again during the intervals between administrations. In the first interval the pressure rose from 83 to 108 mm.; in the second interval from 85 to 122 mm.; in the third interval from 83 to 112 mm. It is the usual experience, when the mechanism of the heart is normal, to find little or no alteration in brachial blood pressure during digitalis administration. Whether this is true when the auricles are fibrillating, is unknown. The fractional blood pressure curve in this patient taken either by palpation or auscultation is unsatisfactory, so that the record of its behavior is not valuable. Nor is it possible to construct a brachial curve based on the levels at which about 40 beats were heard or felt because the counts due to the long examination made necessary by the fractional method are inaccurate. These observations permit us to state merely that we observed not a rise but actually a fall in digital pressure during digitalis administration. The subject requires further study.

SUMMARY.

The function of the arteries as an elastic reservoir between the heart and the capillaries is reviewed. The appropriateness of selecting the exit from this reservoir as the point for estimating its effective pressure is shown. The technique for taking the pressure here by the method of Gaertner is described and its advantage and certain apparent disadvantages are indicated.

The technique of Gaertner is shown to be especially applicable to the study of the blood pressure in fibrillation of the auricles. The use of this technique has brought out a defect in the so called fractional method of taking the pressure in this condition; the brachial and digital curves cross.

Taking pressure of both brachial and digital arteries has shown that certain different types exist; first, that in which both central and peripheral pressures are stable; second, that in which the more central pressure is stable and the peripheral pressure fluctuates; and third, that in which both pressures fluctuate together.

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THE PERIPHERAL BLOOD PRESSURE IN FIBRILLATION OF THE AURICLES.

By ALFRED E. COHN, M.D., and CHRISTEN LUNDSGAARD, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

PLATE 11.

(Received for publication, January 22, 1918.)

In a study of the blood pressure in persons whose auricles were fibrillating we found that satisfactory readings can be obtained at the digital artery by the method of Gaertner (1). It is well known that the pressure in the brachial artery in auricular fibrillation is difficult to take either by the auscultatory or palpatory method on account of the large differences in pulse pressure of succeeding beats. The method of taking the pressure at the digital artery is more likely to be satisfactory, because the pulse pressure is much smaller at this point and the fluctuations from beat to beat, compared with those in the brachial artery, are probably insignificant.

EXPERIMENTAL.

To determine this point we performed the experiments reported below. Dogs were anesthetized with morphine, paraldehyde, and ether. The right femoral artery was connected with a membrane manometer; the small artery on the dorsum of the left hind foot, with a mercury manometer. Two serrefines, to each of which insulated copper wire was soldered, were fastened to the right auricular appendix. They were introduced into the chest through a small intercostal incision. The other ends of the wires were attached to the secondary coil of an inductorium. The auricles were made to fibrillate by stimulating them with an interrupted current, and the action of the ventricles thereupon became completely irregular.

The curves which we obtained show that when the auricles were in a state of fibrillation, the rate of the ventricles rose, in this case from 123 before the inception of this rhythm to 210 (Fig. 1). The

pulse pressure in the right femoral artery fluctuated through a wide range. But in the artery of the dorsum of the left hind foot the fluctuations in pulse pressure were small and did not vary by more than a few millimeters of mercury. It is clear, therefore, that the pressure here does not vary through so wide a range as in the larger vessels; the value of any one beat differs but slightly from that of the others. In view of the small pulse pressure, the systolic and diastolic limits are not far removed from the mean pressure. The readings are simply and directly made. The experiments show, therefore, that direct readings, not far removed from mean pressure, may be obtained in dogs from the small arteries. In man the pressure conditions in the small arteries, like the digital, are doubtless similar to those in the dog. With a technique such as that recommended by Gaertner, satisfactory estimates of pressure should accordingly be possible. Such estimates have been made and the results obtained are published elsewhere (1).

Our experiments bring out, in addition, certain points bearing on the blood pressure when the auricles fibrillate. In the curve reproduced, the fact appears that the general level of the pressure in the smaller vessel does not fall, but on the contrary tends, on the whole, to rise slightly. A similar rise, probably temporary, was observed in three of the four experiments; the pressure fell in only one. In two of the three instances in which the pressure rose at first, it fell later in the experiment, after prolonged anesthesia and operative manipulation. The behavior of the pressure during experimental auricular fibrillation is of interest to us as it was to Lewis (2), because of the bearing of the experimental data on the phenomena in man. Lewis observed that "the disturbances of the circulation are so profound in the human subject, when this curious disorder of the heart's action begins" that he was impelled on this account to investigate the nature of the new conditions experimentally. We too have seen profound disturbances in the circulation under these conditions, but there has, on the other hand, been abundant opportunity to observe instances in which fibrillation of the auricles set in without subjective sensations in the patient. These cases may find their explanation in those of our experiments in which the general level of pressure and the range of pulse pressure did not change in the smaller vessels. This is im-

portant because the maintenance of the pressure level at this location is one of the functions on which the sufficiency of the circulation ultimately depends. If hearts actually undergo this violent change in mechanism without necessary change in pressure, which our experiments show is possible, the fact that patients undergo the same change without sensation is explicable. That the change is accompanied by severe reaction in many individuals is, of course, well known, and in them, as Lewis and we ourselves found, temporary fall in pressure is easily assumed. The influence of ventricular rate, as Lewis has pointed out, is an important factor under experimental conditions. How important it is within the limits in which it fluctuates in patients we are not in a position to say.

SUMMARY.

1. Experiments on dogs show that whereas the pulse pressure in the larger arteries (femoral) varies extensively, it varies within narrow limits only in the small ones (dorsal artery of the foot).

These results supply an experimental explanation for the fact that in man uniform pressure readings were obtained by Gaertner's method at the digital artery.

2. The experiments show likewise that in certain instances the level of pressure is maintained when fibrillation of the auricles sets in.

It is therefore clear that when the mechanism of the heart beat in man changes to fibrillation of the auricles, a change, that is a fall, in pressure need not necessarily develop.

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EXPLANATION OF PLATE 11.

FIG. 1. The effect of fibrillation of the auricles on the pulse pressure in the right femoral artery and in the dorsal artery of the left hind foot. From above down the figure records the signal of stimulation of the right auricle, the blood pressure of the right femoral artery taken by a membrane manometer, the blood pressure from a dorsal artery of the left hind foot, the time in seconds.

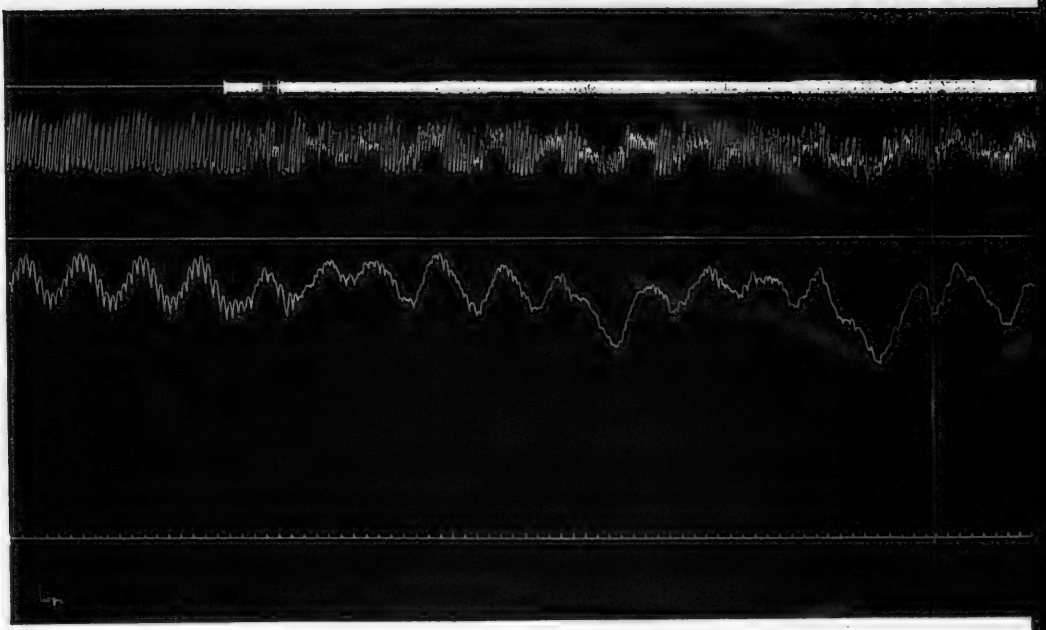
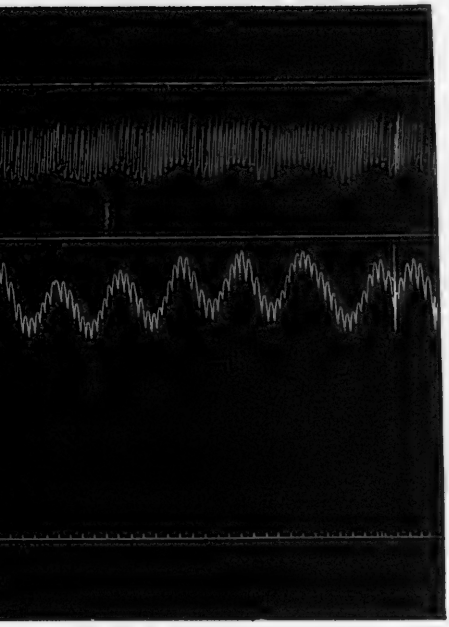


FIG. 1.

FIG. 1.

PLATE 11.



(Cohn and Lundsgaard: Peripheral blood pressure.)

FREE ANTIGEN AND ANTIBODY CIRCULATING TOGETHER IN LARGE AMOUNTS (HEMAGGLUTININ AND AGGLUTINOGEN IN THE BLOOD OF TRANSFUSED RABBITS).*

By PEYTON ROUS, M.D., and OSWALD H. ROBERTSON, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 12 and 13.

(Received for publication, February 28, 1918.)

A singular phenomenon, hitherto undescribed, may frequently be observed in the shed blood of rabbits rendered plethoric by repeated small transfusions from compatible donors.[1] In fresh slide preparations the red corpuscles begin almost at once to clump into masses, and within a few moments the separation of plasma and cells is complete. The blood film, homogeneous at first to the naked eye, is transformed into a mixture of clear fluid and large red flakes. In defibrinated blood allowed to stand at room temperature, the cells fall out rapidly as a red granular sediment, which, in the course of a few hours, may become a solid mass that cannot be broken up without hemolysis. The clumping can occur *intra vasam*, as may be shown by inducing stasis in the rabbit's ear with a tourniquet applied at the base. When the marginal ear vein is opened after ½ hour of such stasis, the blood flowing forth is seen to consist of numerous rather large, dark red flakes in a clear fluid. Under ordinary conditions, the clumping is plainly an extravascular phenomenon, being first

* Read at a joint meeting of the American Association of Immunologists and the Society for Serology and Hematology, New York, April 6, 1917.

[1] The method of transfusion has already been described (Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1917, xxv, 665). The rabbits received intravenously, 6 days in 7, 10 cc. of whole citrated rabbit blood obtained by the cardiac aspiration of compatible donors. For each recipient a series of donors were employed in rotation. Their compatability had been determined by the method of Rous and Turner, *J. Am. Med. Assn.*, 1915, lxiv, 1980.

visible in slide preparations some 12 to 40 seconds after the blood is drawn.

Protection of the Organism.

The clumping of the red cells would result in pulmonary emboli and be quickly fatal, were it not in some way prevented *in vivo*. Our first tests had to do with this phenomenon. The problem presented proved unexpectedly easy of solution. The clumping is absolutely conditioned by temperature. If no precautions are taken to keep warm the rabbit's ear when bound with a tourniquet, clumping occurs in the vessels, as already described. But if the bound ear is kept in water at the body temperature, the blood taken from it after half an hour shows no clumping, and this appears only secondarily as cooling occurs. Again, if a little fresh blood is taken into each of two thick-walled glass tubes of capillary bore, one of which has been chilled in the ice box, the other heated to body temperature, a gross clumping will be seen almost at once in the cold tube, whereas in the warm one the blood remains homogeneous during the 3 or 4 minutes before clotting takes place. Blood allowed to drop directly into a few cubic centimeters of cold salt solution shows clumping almost before it can be distributed in the medium; whereas if the solution has the body temperature, the corpuscles remain separate for 24 hours even—the longest time over which we have observed them. If, after 24 hours of warmth, the mixture is cooled in running water, clumping occurs in case the initial dilution of the blood has not been too great.

The Clumping Is a True Agglutination.

The character of the temperature control suggests that the clumping is caused by hemagglutinins. Landsteiner has shown that the isoagglutinins and the weak normal autoagglutinins of several animal species, among them the rabbit, are similarly governed by temperature and have most effect in the cold.[2] The clumping, viewed microscopically, has certainly the appearance of a true agglutination, while the circumstances of its occurrence are such as would favor the development of agglutinins. It is most pronounced when the rabbit

[2] Landsteiner, K., *Münch. med. Woch.*, 1903, l, 1812.

has received from ten to fifteen transfusions. After the first five or six, a change begins to be noticeable in the shed blood. Rouleau formation is more marked than normally. Then, as the injections are continued, the rouleaux of the shed blood, which are strikingly long and perfect, tend to draw together into masses in which they undergo little disorganization (Figs. 1 and 2). Finally, the tendency to clumping becomes so strong that the rouleaux present when the blood is first shed collapse after a few moments into irregular agglomerates of corpuscles lying against one another without definite arrangement. In slide preparations these masses are at first connected by large trunks of cells, but shrinkage soon takes place—doubtless from closer apposition of the cells—and the trunks pull out into thin strands, often only one or two cells thick (Figs. 3 and 4). Within the large plasma spaces thus opened up, there are almost no corpuscles, red or white. When pressure is put on the cover-glass the cells are often stretched into long ropes, but they hold together tenaciously as if made of a sticky, elastic material. When the pressure is released they resume their normal shape.

If the clumping is caused by a true agglutinin, a separation of this element from the cells should be possible. It has been accomplished by repeatedly washing the cells in warm salt solution in which, as already stated, they are not clumped. Cells thus washed in the centrifuge remain unclumped when sedimented and cooled. But they at once clump when placed in serum obtained from a specimen of the blood defibrinated and centrifuged in the warm.

Attempts were now made to obtain the agglutinin in salt solution by the method Landsteiner employed with the weak, normal auto-agglutinin of rabbits.[2] Landsteiner allowed a small quantity of cells in a large quantity of serum to stand over night on ice. Complete agglutination took place. The cell mass was now washed several times in ice cold salt solution and then placed in a little of the fluid at body temperature. After some hours it was centrifuged while still warm. The heating had liberated the agglutinin, which passed into the salt solution, and the latter now possessed the ability to agglutinate cells.

In the case of our transfused rabbits the serum factor responsible for clumping was so strong that there was no necessity for the serum

to preponderate greatly over the cells or for more than a brief chilling and warming.

Experiment 1.—A little of the blood of a transfused rabbit was taken into a test-tube surrounded by a water jacket at 38°C. and was defibrinated with glass beads. From this 0.6 cc. was pipetted off and cooled in ice. The corpuscles rapidly clumped and fell to the bottom of the tube. After 45 minutes centrifugation was done in an ice jacket. The serum was immediately pipetted off and kept. It will be termed Serum A. The cells, which had formed a solid mass, were now twice washed with 3.5 cc. of ice cold salt solution. The cell mass showed no tendency to break up when thus handled. All fluid was now pipetted away, 0.3 cc. of fresh salt solution put on, and the tube transferred to a water bath at 40°C. Within 5 minutes the mass had broken up into a homogeneous cell suspension. After 10 minutes more, an attempt was made to throw down the cells while still warm, but though a warm water jacket was used, the temperature fell sufficiently during the process for some clumping to occur. The tube was therefore warmed again for 15 minutes and again centrifuged rapidly, but now in a jacket of warm paraffin oil. This time the heat was retained, no clumping occurred, and the fluid—Fluid B, as it will be called—was immediately taken off for test. The cell sediment was then twice washed in an excess of warm salt solution and made up to the original blood bulk. The cells remained unclumped.

The following mixtures were now set up:

(*a*) 1 part cell suspension + 3 parts Fluid B.
(*b*) 1 part cell suspension + 3 parts Fluid B + 9 parts salt solution.
(*c*) 1 part cell suspension + 3 parts Serum A + 9 parts salt solution.
(*d*) 1 part cell suspension + 12 parts salt solution.

In (*a*) marked clumping of the cells took place almost at once at room temperature. The other three mixtures were cooled in ice. Strong clumping was observed after a few minutes in (*b*), slight clumping in (*c*), and none at all in (*d*). From the presence of a slight clumping in the mixture (*c*) it is evident that the factor responsible for agglutination was not completely removed from the serum when the defibrinated blood was cooled to 0° C.

Variation with Temperature.

In experiments such as the foregoing, success was obtained only after the necessity for careful maintenance of the essential temperatures had been recognized. During the separation of the agglutinin a moment's accidental cooling or warming was sufficient to fix or liberate it in large part from the cells, thus leading to confusion. When blood was defibrinated in the warm and then gradually cooled in tubes that permitted of microscopic inspection, slight agglutination was

found to appear as the temperature fell from 36° to 35°C. At 33°C. large clumps formed; and at room temperature (22°) the agglutination was massive. When the tube was warmed again, all clumping disappeared at between 35° and 36°C. In the experiment for the separation of agglutinin given in detail above, all agglutinin was not absorbed from the serum at 0°C. This may have been due to insufficient contact of serum and cells owing to the rapid clumping and sedimentation of the latter when suddenly chilled. We have repeatedly noted that a potent serum, if allowed to separate from the clot at room temperature, may contain no agglutinin whatever.

Reversibility of the Reaction.

The rapid variation in the clumping with changes in temperature has led us to investigate the reversibility of the agglutination. A sample of blood was defibrinated in the warm, as usual, filtered through gauze, and placed, first on ice, and, when clumping was complete, in water at body temperature. This was repeated as fast as massive clumping or its reverse, complete dissociation, had occurred. After nine coolings and warmings, the cells still clumped and separated as rapidly and completely as at first. There was an entire absence of the gummy change seen when cells are repeatedly clumped by a heteroagglutinin.[3]

Strength of the Agglutinin.

The great variation in the clumping at different temperatures and the rapidity with which the agglutinating principle is fixed or freed has rendered difficult a precise determination of its strength. We have employed a crude method, allowing the blood to fall from the rabbit's ear, drop by drop, into known quantities of warm salt solution, and noting the agglutination when the mixtures have been cooled for some minutes at room temperature. Under these circumstances the dilution of both antigen and antibody vary, but they vary alike, maintaining practically a constant relation to each other. There is not the same likelihood of error in the ingredients as when mixtures are made of cells and serum separated from each other in the warm.

[3] Landsteiner, K., and Reich, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1905, xxxix, 83

But the temperature of the salt solution must be above 37°C., since even slight cooling results in some clumping of the cells before they can be properly distributed.

The strongest agglutinin found in the transfused rabbits caused well defined clumping in a mixture of one drop of blood with 100 cc. of salt solution; that is, in a plasma dilution of approximately 1: 2,800.[4] No clumping occurred in the 1: 5,600 mixture. The plasma of a second rabbit agglutinated the cells when diluted 500 times. These were exceptional instances. In the majority of cases the plasma failed to clump the cells when it was diluted with more than 20 parts of salt solution.

The clumping phenomenon did not regularly appear in transfused rabbits. Indeed, in ten out of twenty transfused with a special view to its development it was never observed despite the fact that the transfusions were continued far beyond the usual period. Furthermore, in rabbits transfused persistently any agglutinating factor that had developed tended to disappear. A similar disappearance of precipitin following unduly prolonged immunization has been recorded by Tchistovitch[5] and Nuttall.[6] Sudden reductions in the plethora of the recipients, accomplished by bleeding, failed to induce or increase the clumping phenomenon, as did also a use of donors with cells agglutinable by the recipient's plasma.

Agglutination and Anemia.

The peculiar temperature control of the agglutination in the transfused animals has an obvious, if superficial, likeness to that occurring in paroxysmal hemoglobinuria. And the fact that the animals with the strongest agglutinin developed a sudden anemia, in the midst, so

[4] The percentage volume of the blood plasma was reckoned from a comparison of the rabbit's hemoglobin with that of normal rabbits of which the cell plasma ratio had been established with Epstein's hematocrit. In these normal animals the cells averaged 42 per cent and the plasma 58 per cent of the blood volume. Twenty drops of blood were assumed to make 1 cc. This was the case in actual tests.

[5] Tchistovitch, T., *Ann. Inst. Pasteur*, 1899, xiii, 406.

[6] Nuttall, G. H. F., Blood immunity and blood relationship, Cambridge, 1904, 127.

to speak, of their plethora, has in this respect a special interest. The hemoglobin of the rabbit with an agglutinin active in a 1:2,800 serum dilution fell after the fifteenth transfusion from 128 (Sahli) to 75 per cent in the course of 4 days, despite the injection on each of these days of the usual 10 cc. of blood. The transfusions were now stopped, and the hemoglobin fell to 37 per cent in 2 days more, after which a gradual recovery ensued. The animal at no time manifested symptoms of distress. Some of the blood changes in this rabbit and others of like sort have already been described by Robertson in another connection.[7] In these instances the hemagglutinin was at its greatest strength when the anemia developed, while in animals with a weak agglutinin or none, an anemia was never observed, but, on the contrary, plethora was maintained for weeks after the transfusions had been stopped.

No adequate search has yet been made for an hemolysin in the plasma of the animals becoming anemic, but we have chilled, without result, two plethoric rabbits possessing a weak agglutinin (active in a 1:5 dilution of the whole blood) in the hope of initiating a drop in the hemoglobin. The chilling was accomplished by means of ice cold water, in which the well shaved ear of the rabbit was submerged for $\frac{1}{2}$ to 1 hour. Throughout this period the circulation in the cold ear was exceptionally good. The rectal temperature fell to 37°C., considerably below the normal for the rabbit, but not low enough to produce the *in vitro* agglutination of blood corpuscles.

Persistence of the Agglutinin.

The agglutinating principle, once it has appeared in a blood, persists for a long period and is relatively uninfluenced by the disappearance of plethora, or by sudden intercurrent anemia of the sort just described, or by moderate bleedings. In a rabbit, for example, in which the hemoglobin fell from 125 to 27 per cent in the course of a few days, with a gradual return to the normal of 90 per cent, the agglutinin persisted throughout. 110 days after the normal hemoglobin had been finally reached, the blood still showed clumping when di-

[7] Robertson, O. H., *J. Exp. Med.*, 1917, xxvi, 221.

luted with two volumes of salt solution. This was 133 days after the last transfusion.

SUMMARY.

In rabbits transfused almost daily with the whole citrated blood of other rabbits, an extraordinary condition often develops, which manifests itself in an almost immediate clumping together of all the red cells in specimens of the shed blood. This clumping is due to one or more true agglutinins, of which the strength may be such as to cause clumping in a 1: 2,800 plasma dilution.

The agglutinating principle circulates with the corpuscles against which it is effective; but under ordinary circumstances intravascular clumping fails to occur because the union of antigen and antibody can take place only at a temperature several degrees below that of the body. If the temperature is sufficiently lowered, as when a tourniquet is applied to the rabbit's ear, intravascular clumping ensues. In defibrinated blood, gradually cooled, clumping is first noted as the temperature of 35°C. is approached; and at room temperature (22°) the corpuscles will often come together in a short time into a single, solid mass. At 0°C. the agglutination is still more marked. The reaction seems to be completely reversible, for when the blood is warmed again, the clumps break up and disappear at between 35° and 36°C. Cooling and warming with the resultant clumping and dissociation can be carried out many times on the same blood specimen without apparent change in the corpuscles or in the rapidity of the reaction. The response to temperature changes is extremely prompt.

Once it has been elicited, the agglutinating principle may persist for a long time after the transfusions are stopped. In one instance it was still strong 133 days after the last transfusion. During this period the plethora was succeeded by a severe anemia, which in turn was recovered from. In many rabbits no agglutinin develops, and a continuance of the transfusions will not elicit it. Indeed, when present it tends to disappear if the transfusions are persisted in.

In several of the animals in which the agglutinin was strongest, the plethora was suddenly succeeded by severe anemia, despite continued transfusions. The character of the temperature control of

the agglutination, which somewhat resembles that of the hemolysin in paroxysmal hemoglobinuria, has led us to consider whether the blood destruction might not be due to accidental chilling of the animal. Efforts to induce a fall in the hemoglobin by placing the rabbit's ear in ice water have as yet been unsuccessful. Thus far no adequate search for an hemolysin has been made.

The object of the present paper has been to describe a condition in which large amounts of free antigen and antibody circulate together in the organism, and to demonstrate the factor which prevents their union, the results of which could easily be fatal. The causes of the condition will be dealt with in a subsequent communication.

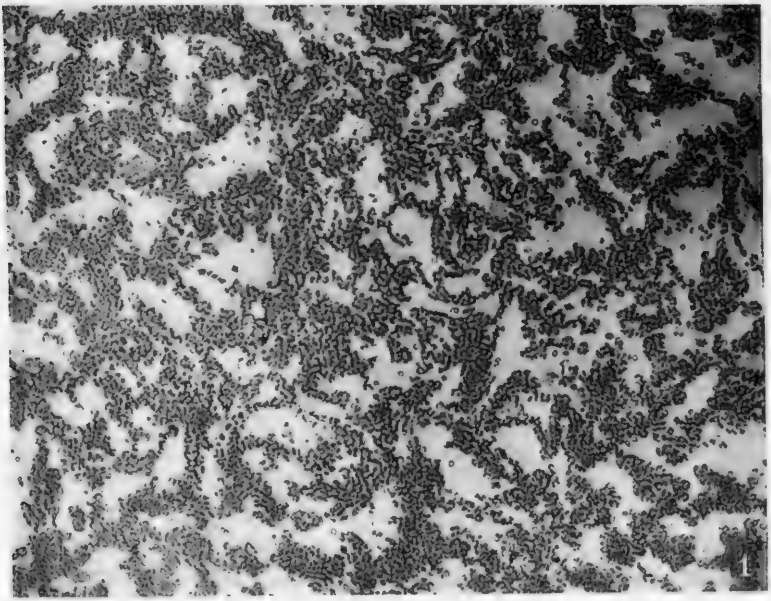
EXPLANATION OF PLATES.

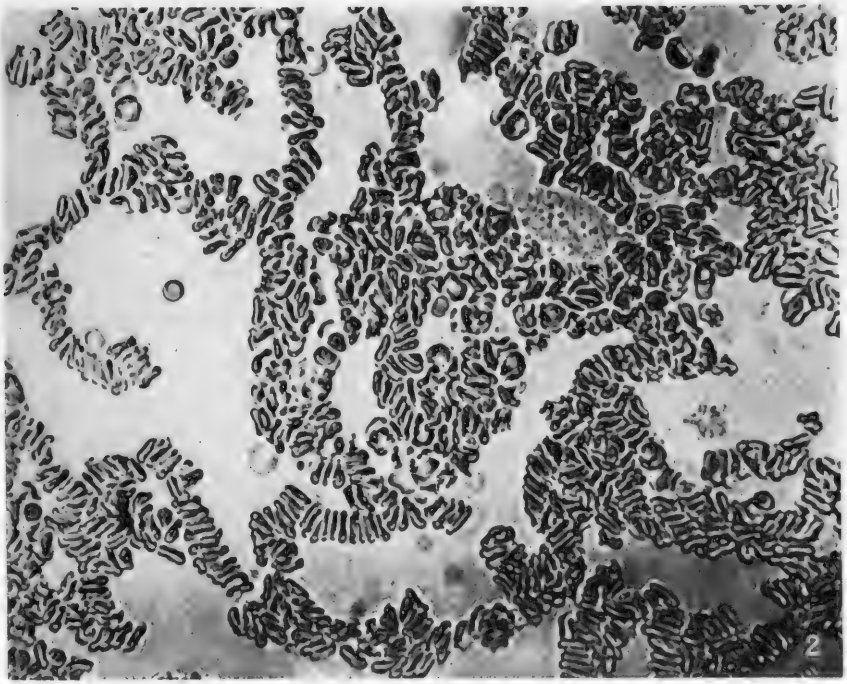
PLATE 12.

FIG. 1. A weak clumping phenomenon. The rouleaux are largely intact.
FIG. 2. A weak clumping phenomenon. Marked rouleau formation.

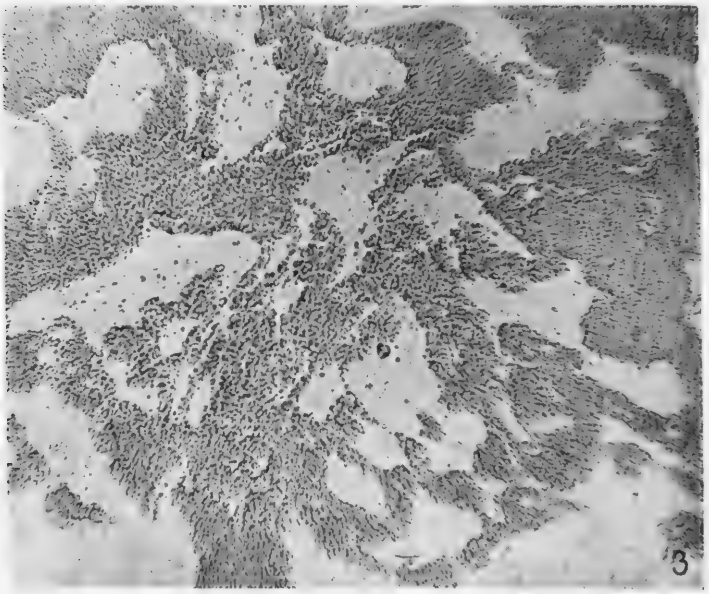
PLATE 13.

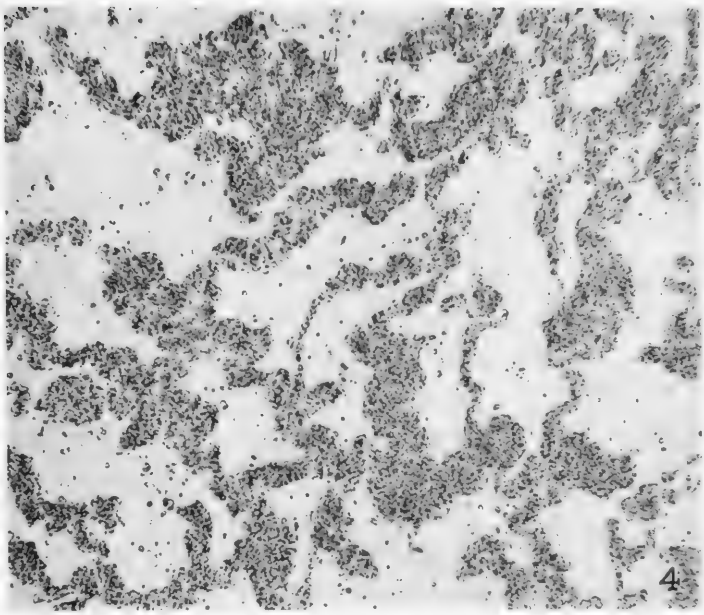
FIGS. 3 and 4. The clumping phenomenon in pronounced form. In the large serum spaces there are almost no free cells.





(Rous and Robertson: Free antigen and antibody.)





(Rous and Robertson: Free antigen and antibody.

A STUDY OF ACUTE MERCURIC CHLORIDE INTOXICATIONS IN THE DOG WITH SPECIAL REFERENCE TO THE KIDNEY INJURY.*

By WILLIAM deB. MacNIDER, M.D.

(*From the Laboratory of Pharmacology of the University of North Carolina, Chapel Hill.*)

PLATES 14 TO 16.

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A review of the relatively scant literature dealing with acute mercuric chloride intoxications shows a preponderance of clinical papers with suggestions relative to the treatment of poisoning and bu few investigations which have as their object an understanding of the cause of the remote tissue changes, especially those of the kidney. The investigations which have been primarily concerned with the acute pathology induced by mercuric chloride in organs remote from the intestine have either considered the injury to be dependent upon the action of the metal on the vascular tissues of the organ, or to be due to the action of the metal as such on the parenchyma of the organ during elimination.

Von Mehring[1] considered the toxic action of mercury in the kidney and also in the intestine to be due to a general vasomotor paralysis. Heineke[2] and Kaufmann[3] held the opinion that mercury in the blood had a thromboplastic action and that the damage to the kidney depended upon the formation of thrombi with the production of infarcts. Schmiedeberg[4] considers the changes in the

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] von Mehring, J., Ueber die Wirkungen des Quecksilbers auf den thierischen Organismus, *Arch. exp. Path. u. Pharm.*, 1881, xiii, 86.

[2] Heineke, W., Die Fermentintoxication und deren Beziehung zur Sublimat- und Leuchtgasvergiftung, *Deutsch. Arch. klin. Med.*, 1888, xlii, 147.

[3] Kaufmann, E., Neuer Beitrag zur Sublimatintoxication nebst Bemerkungen über die Sublimatniere, *Virchows Arch. path. Anat.*, 1889, cxvii, 227.

[4] Schmiedeberg, O., Grundriss der Pharmakologie in Bezug auf Arzneimittellehre und Toxikologie, Leipsic, 5th edition, 1906.

mucous membrane of the colon and kidney to be due to the destructive action of the metal during its elimination. In a recent study of acute mercury poisoning by Burmeister and McNally[5] the same conclusion is apparently reached as that held by Schmiedeberg concerning the way in which the toxic effect of mercury is induced. The authors note the marked variation in the toxic action of the metal and consider that the hepatic changes vary with the duration of the intoxication, while the kidney damage varies with the size of the dose as well as the duration of the intoxication. In the animals receiving massive doses immediate renal changes develop which vary with the size of the dose, while with smaller doses the renal changes depend upon the length of time the animal is able to withstand the intoxication.

In an investigation[6] conducted several years ago, in which various nephrotoxic agents were employed, a study was made of the relative affinity of different kidney poisons for the epithelium of the kidney and of the relation between the degree of epithelial damage and the ability of the kidney to form urine. Mercuric chloride was used in eight animals. The salt was given subcutaneously in the dose of 10 mg. per kilo. The nephropathy induced by these injections was variable in both the frequency with which it occurred and the constancy of the pathological changes in the kidney. Two of the animals became rapidly anuric and showed an extensive necrosis of the renal epithelium, especially that of the convoluted tubules. The remaining animals either showed no toxic effect from the injections, or after a short period of albuminuria made a complete recovery.

Recent studies[7,8] of the acute nephropathy induced in dogs by uranium have shown a similar variation in the toxicity of this metal for the kidney. These studies have furthermore shown that when the functional capacity of the kidney has been reduced by uranium the vascular mechanism retains its responsiveness to various peripherally acting stimuli comparable in degree with that of the normal organ. The lack of functional response has been associated with a variable amount of degeneration of the renal epithelium.

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[5] Burmeister, W. H., and McNally, W. D., Acute mercury poisoning. A parallel histological and chemical study of the renal and hepatic tissue changes as compared with the rapidity of absorption and the amount of mercury present in the circulating blood at the time such changes occur, *J. Med. Research*, 1917, xxxvi, 87.

[6] MacNider, W. deB., A study of the renal epithelium in various types of acute experimental nephritis and of the relation which exists between the epithelial changes and the total output of urine, *J. Med. Research*, 1912, xxi, 79.

[7] MacNider, A study of the action of various diuretics in uranium nephritis, *J. Pharm. and Exp. Therap.*, 1911–12, iii, 423.

[8] MacNider, The vascular response of the kidney in acute uranium nephritis; the influence of the vascular response on diuresis, *J. Pharm. and Exp. Therap.*, 1914–15, vi, 123.

In later papers,[9,10] the observation was made that in acute uranium intoxications the severity of the epithelial damage in the kidney shows a correlation with the degree of acid intoxication induced by the metal, and, furthermore, that the damage to the kidney may in large measure be prevented by the intravenous injection of an alkaline solution.

With these observations in mind the present study of the toxicity of mercuric chloride has been undertaken with the object of ascertaining the cause of the kidney damage and with the hope that some therapeutic agent might be developed that would protect the kidney against the toxic effect of this metal. A preliminary note of this work has recently appeared.[11]

EXPERIMENTAL.

Dogs were used in the experiments which furnish a basis for this study. The animals were placed in metabolism cages, given 500 cc. of water daily by stomach tube, and fed on bread with a small amount of cooked meat. The urine was collected from the cages twice a day. Females were catheterized at the second collection of urine in order to obtain an accurate record of the total output in a 24 hour period. Forty-three of the fifty-six animals used were females. The animals were kept under observation for 3 days prior to administering the mercury. During this normal period (Table I) and following the use of the mercury the animals were studied as follows: Hydrogen ion determinations of the blood were made by the indicator method of Levy, Rowntree, and Marriott.[12] The alkali reserve of the blood and determinations of the carbon dioxide tension of alveolar air were made

[9] MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

[10] MacNider, The efficiency of various diuretics in the acutely nephropathic kidney, protected and unprotected by sodium carbonate. II, *J. Exp. Med.*, 1917, xxvi, 19.

[11] MacNider, A study of the acid-base equilibrium of the blood in acute bichloride intoxications, *Proc. Soc. Exp. Biol. and Med.*, 1917, xiv, 140.

[12] Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen-ion concentration of the blood, *Arch. Int. Med.*, 1915, xvi, 389.

by the methods of Marriott.[13,14] The blood urea determinations were made by the method of Marshall,[15] following the modification suggested by Van Slyke and Cullen.[16] The phenolsulfonephthalein test for kidney function was conducted according to the technique devised by Rowntree and Geraghty.[17] Quantitative albumin determinations were made by Esbach's method, and the percentage of glucose in the urine was estimated by Benedict's reagent. The quantitative determinations of acetone were made by Folin's[18] method as modified by Hart.[19] During the course of the experiments it became necessary to ascertain the relation, if any existed, between the elimination of mercury by the kidney and the development of the acute kidney injury. In the absence of satisfactory quantitative tests the recently devised qualitative test of Elliott was used.[20] The test is both simple and very delicate, and may be employed in a relatively quantitative fashion by noting the amount of mercury deposited on gold leaf as an amalgam from a series of urines.

In order to induce an experimental condition comparable with that obtained when mercuric chloride is taken accidentally or for suicidal purposes, the poison was introduced by a stomach tube. The animals were first given hypodermically 0.25 cc. of a 4 per cent solution of morphine sulfate. After the initial excitement and emesis induced by the morphine, the animals became partially narcotized. During

[13] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[14] Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

[15] Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

[16] Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

[17] Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909–10, i, 579.

[18] Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

[19] Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

[20] Elliott, J. A., A new and delicate method for the detection of mercury, *J Am. Med. Assn.*, 1917, lxviii, 1693.

this period of narcotization the animals were given 15 mg. of mercuric chloride per kilo. A 1 per cent solution was used. At the time of administration the dose of mercuric chloride was made up to a volume of 100 cc. with distilled water. As a result of the depressed irritability of the animals and the dilution of the dose of the poison, the irritant effect of the mercury was sufficiently modified to prevent vomiting in the majority of the animals for a period of 4 hours. Eight of the animals vomited the mercury within an hour after its use. The rest of the animals retained the poison for 4 hours or longer. Excluding the eight animals referred to above, the poison was unquestionably retained long enough for it to be absorbed and induce its remote toxic effect.

All the animals receiving mercury developed a gastroenteritis which varied widely in severity and duration. This variation apparently depended upon the total amount of the poison received by the animal and the length of time which elapsed between the administration of the poison and the commencement of vomiting. An analysis of the experiments, from the standpoint of the final outcome of the intoxicated animals, permits their classification into four groups. The first group is represented by eight animals (Table II, Group I). The animals of this group developed an intense gastroenteritis which was characterized by persistent vomiting of large quantities of fluid and by frequent, bloody, mucous stools. The animals died in a state of collapse within 48 hours after receiving the poison.

The second group of animals (Table III, Group II) also showed a severe local reaction from the mercury. The vomiting and stools were frequent. In this series the gastroenteritis showed a tendency to subside during the first 3 days of the intoxication. All the animals died within 7 days from the commencement of the intoxication. Six died in convulsions. The remaining four animals died in air-hunger.

The third group of animals (Table IV, Group III) showed a moderately severe gastroenteritis. During the subsidence of the gastroenteritis, or several days after the symptoms of this condition had disappeared, the animals showed a beginning acid intoxication. The hydrogen ion determinations of the blood were variable. The reserve alkali of the blood, however, showed a depletion and the tension of alveolar air carbon dioxide was reduced. These changes, indicative

of a beginning acid intoxication, persisted from 1 to 8 days. The twenty animals forming this group made a complete recovery in as far as any immediate effect from the mercuric chloride intoxication was concerned.

The remaining group of eighteen animals (Table V, Group IV) showed a gastroenteritis which was variable both in severity and duration. In all the animals this symptom of poisoning disappeared. Following the subsidence of the enteritis, and in three of the experiments as late as the 9th day, the animals either gradually or rapidly developed an acid intoxication, and, depending upon the severity and duration of this intoxication, became anuric. Seven of the animals died in air-hunger. From the foregoing outline of the variation in the toxicity of mercuric chloride as shown by the animals comprising the different groups, it becomes necessary to analyze the effect of the poison in the various groups with the object of ascertaining the cause of the variation and the way in which the toxic action of the metal is induced.

Observations on Normal Animals.

The following observations on normal animals extended over a period of 3 days. The results have been recorded in Table I. The number of the experiment in this table corresponds with the number

TABLE I.

Observations on Normal Animals.

Group No.	Experiment No.	Urine.	Albumin, glucose, acetone.	Phenolsulfonephthalein.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.
		cc.		*per cent*	*per cent*			*mm.*
I	1	418	0	69	0.012	7.35	8.05	45
I	2	330	0	83	0.021	7.35	8.1	45
I	3	381	0	79	0.013	7.35	8.1	43
II	4	681	0	81	0.014	7.5	8.1	44
II	5	492	0	80	0.012	7.55	8.0	42
II	6	891	0	73	0.012	7.45	8.1	43
III	7	271	0	84	0.013	7.3	8.05	41
III	8	508	0	82	0.012	7.45	8.1	45
III	9	320	0	86	0.012	7.35	8.0	42
IV	10	381	0	71	0.012	7.45	8.1	42

of the animal after it had received the poison. The observations on the various groups of intoxicated animals will be found in Tables II to V. A study of the normal findings contained in Table I shows that all the animals were freely diuretic, and that the urine was free from albumin, glucose, and acetone bodies. The centrifugalized urines did not show casts. The appearance of phenolsulfonephthalein in the urine was not delayed longer than 10 minutes. The total output of the dye in a 2 hour period varied between a minimum output of 69 per cent and a maximum output of 86 per cent. The percentage of blood urea in all the animals remained very constant, varying between 0.012 and 0.021 per cent. The hydrogen ion concentration of the dialyzed whole blood prior to aeration varied between 7.35 and 7.55. After the aeration of the dialysate the reserve alkali showed slight variation, 8 to 8.1. The determinations of the tension of alveolar air carbon dioxide showed a constancy with that of the reserve alkali determinations. In as far as the urine, renal function, and acid-base equilibrium of the animals were investigated, the animals of all the groups were normal.

Observations on the Different Groups of Animals Intoxicated by Mercuric Chloride.

Group I.

The first group of experiments (Table II) is represented by the animals which died from the intoxication within 48 hours after receiving the mercury. All the animals showed a clinical condition comparable with the state of shock and collapse which is obtained in man from the use of a concentrated corrosive poison. They were unable to stand. The surface of the body was cold, the tongue and gums were cyanotic, the respirations shallow, and the heart beat was fast with feeble heart sounds. The pupils in four of the animals were widely dilated for several hours before death. The animals of this group had an intense gastroenteritis, as was indicated by the persistent vomiting of fluid, in two instances streaked with blood, and by the frequent, bloody, mucous stools. The autopsies showed the usual effect of a strong corrosive on the mucous membrane of the stomach and intestine.

TABLE II.

Group I. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools	Vomitus.	Condition of animal
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
1	15	1	790	0	0	0	75	Tr.	0.012	7.45	8.05	42	Severe enteritis. Blood.	Frequent.	Bad.
		2	0	0	0	0	0	0	0.027	7.35	8.0	38	No change.	No change.	Died in collapse.
2	15	1	70	Tr.	Tr.	0	77	Tr.	0.040	7.35	8.0	39	Severe enteritis. Blood.	Frequent.	" " "
3	15	1	130	"	0.39	4.612	65	"	0.061	7.3	7.9	37	" " "	"	" " "

A review of Table II, containing representative experiments from this group, shows that the output of urine is rapidly reduced. This reduction is not associated with a comparably great reduction in the elimination of phenolsulfonephthalein. There is no marked retention of blood urea. In Experiment 2, Table II, the animal formed only 70 cc. of urine in the first 24 hour period after receiving the mercury. The elimination of phenolsulfonephthalein was 77 per cent. In Experiment 3 the animal formed 130 cc. of urine in a similar period with a phenolsulfonephthalein elimination of 65 per cent. There is in this group a lack of correlation between the ability of the kidney to form urine and to secrete phenolsulfonephthalein. The urine has been either free from albumin or has contained a mere trace. In only one animal was glucose present in the urine. The urine of this animal also showed a trace of acetone, 4.6120 mg. per 100 cc. In this group of animals only a slight disturbance in the acid-base equilibrium of the blood occurred. In Experiment 2, Table II, at the end of the first 24 hours of the intoxication the alkali reserve was 8 as compared with a normal reading of 8.1, and the tension of alveolar air carbon dioxide was 39 mm. as compared with the normal of 45 mm. The animal died of shock 6 hours later. Experiment 3, Table II, shows the greatest disturbance in the acid-base equilibrium of any of the members of the group. Associated with the reduction in the reserve alkali of the blood the elimination of phenolsulfonephthalein is reduced from a normal of 78 per cent to 65, and both glucose and acetone appear in the urine. At this early stage of the intoxication only a trace of mercury was found in the urine.

The animals comprising Group I die in collapse which is apparently dependent upon the severity of the local corrosive action of the mercury in the stomach and intestine. Death occurs before sufficient time has elapsed for the development of the kidney injury. The reduction in the output of urine is probably dependent upon a disturbance in the functional capacity of the vascular mechanism of the kidney induced by the deflection of arterial blood away from the kidney to the splanchnic viscera. The absence of degenerative changes in the kidney and the presence of an intense congestion of the splanchnic vessels would apparently permit this deduction.

Pathology of the Kidney.—The kidneys of this group of animals

were removed immediately after death. Postmortem changes were eliminated. The kidneys have had a dark cyanotic appearance. On section the organs have shown an engorgement with venous blood which has been especially marked at the corticomedullary boundary zone and has extended into the medulla in the form of streaks outlining the return veins from the venous arches. Histologically the glomerular vessels have shown an engorgement with blood. No exudate or free hemorrhage has been observed in the glomeruli. The endothelial nuclei of the glomerular capillaries have appeared prominent and stained deeply. The epithelium of the tubules, especially that of the convoluted tubules, has shown an early cloudy swelling, consisting in the appearance of albuminous granules in the cytoplasm without much increase in the volume of the cells. The nuclei of the epithelium have shown an increase in size out of proportion to the changes in the size of the cells. No exudate or extravasation of blood has been observed in the intertubular connective tissue. (Fig. 1.)

Group II.

The second group of experiments consists of the animals which first developed a severe gastroenteritis, and then, during the subsidence of the gastroenteritis, developed an acute acid intoxication with an associated anuria. The animals of this group either died in air-hunger or in convulsions.

A review of the experiments representative of this group of animals (Table III) shows that during the 1st day of the intoxication the output of urine was variable. In Experiments 4 and 6 a sharp reduction in the formation of urine occurred, while in the animal of Experiment 5 the output of urine was in excess of the average normal secretion. In all the animals, as the intoxication progressed, the formation of urine rapidly decreased. The amount of albumin in the urine was slight and is no indication of the severity of the kidney damage. Six of the animals of this group showed both glucose and acetone in the urine. The glucose generally appeared in the urine after the appearance of albumin. The appearance of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood. The elimination, however, of these bodies by the kidney does not show a

TABLE III.

Group II. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
4	15	1	312	Tr.	Tr.	14.7321	71	Heavy.	0.016	7.4	8.0	40	Severe enteritis. Blood.	Frequent.	Bad.
		2	418	"	"	4.6371	28	Tr.	0.021	7.3	7.9	32	No change.	Less frequent.	"
		3	121	"	"	3.7241	Tr.	"	0.067	7.2	7.8	20	Less frequent.	Less frequent.	Very bad.
		4	0	0	0	0	0	0	0.078	7.1	7.75	20	No change.	None.	Died in convulsions.
5	15	1	665	0	0	0	35	Tr.	0.015	7.4	7.95	40	Severe enteritis. No blood.	Frequent.	Bad.
		2	31	Tr.	Tr.	Insufficient urine.	0	Insufficient urine.	0.029	7.3	7.85	25	No change.	Occasional.	Very bad.
		4	0	0	0	0	0	0	0.092	7.1	7.75	15	Less frequent. No blood.	None.	Died in air-hunger.
6	15	1	281	0.25	0	0	Tr.	Heavy.	0.033	7.2	7.9	34	Severe enteritis. Blood.	Occasional.	Bad.
		2	31	0.75	Tr.	4.4784	"	Tr.	0.044	7.1	7.55	15	Improved.	None	"
		3	8	1.2	Insufficient urine.	Insufficient urine.	Insufficient urine.	Insufficient urine.	0.081	7.1	7.5	12	No change.	"	Died in air-hunger.

parallel with the reduction in the alkali reserve. With the further depletion of the alkali reserve, and associated with the decreased elimination of phenolsulfonephthalein by the kidney, a decrease in the output of acetone occurs.

The elimination of phenolsulfonephthalein was reduced in all the experiments. The degree of reduction in the output of the dye varied in the different animals. There was no correlation between the quantitative output of phenolsulfonephthalein and the elimination of mercury by the kidney. In Experiment 4 a heavy amalgam of mercury was obtained from the urine of the animal secreted during the 1st day of the experiment, yet the phenolsulfonephthalein output remained high, 71 per cent. In Experiment 5, however, with only a trace of mercury in the urine, the output of phenolsulfonephthalein was reduced from 80 to 35 per cent.

The experiments show a relation between the phenolsulfonephthalein output and the reduction in the alkali reserve of the blood. The animals which showed a rapid depletion in the alkali reserve also showed a sharp decrease in the ability of the kidney to secrete the dye. Following the variation in the output of urine which was noted in the different animals early in the intoxication, as the intoxication progressed all the animals showed a rapid reduction in the formation of urine and became anuric. The anuria in this series of animals was not associated with a condition of shock as in the anuric animals of Group I. With the progressive decrease in the formation of urine the gastroenteritis lessened in severity. Associated with the reduction in the output of urine there occurred a rapid decrease in the elimination of phenolsulfonephthalein, a retention of blood urea, and the development of a severe acid intoxicaton. The animals dying in air-hunger showed the greatest depletion of the alkali reserve of the blood and the most marked decrease in the tension of alveolar air carbon dioxide. In Experiment 5, 2 hours before death the animal had a reserve alkali reading of 7.75 and a tension of alveolar air carbon dioxide of 15 mm. In Experiment 6 the alkali reserve was reduced to 7.5 and the tension of alveolar air carbon dioxide to 12 mm. None of the animals of the group survived longer than 7 days.

Pathology of the Kidney.—The kidneys from this group of animals were either removed immediately after death or the animals were

killed while in air-hunger and the organs secured for the pathological study. The kidneys are pale and swollen. On section the cortex bulges through the cut capsule. The cut surface appears pale and relatively bloodless. The microscopic examination has shown the glomeruli to be in a fair state of preservation. The capillaries are not engorged with blood. The endothelial nuclei are swollen and stain intensely. The epithelium of the tubules, except that of the junctional tubules, has shown a remarkable degree of swelling and early necrosis. The swollen cells in many tubules have not only completely occluded the lumen of the tubule, but give to the tubule an increased transverse diameter. The nuclei are large and hypochromatic. In other tubules the nuclei have disappeared, the cells having undergone complete necrosis. (Fig. 2.)

Group III.

The third group of experiments consists of the animals which received mercuric chloride and, after recovering from the mercury enteritis, developed an acid intoxication and later made a complete recovery. In this group of animals which did not develop the severe gastroenteritis characteristic of the early stage of the intoxication in the two previous groups, the output of urine was not greatly reduced (Table IV). Albumin was present in the urine of all the animals with two exceptions. The amount did not exceed 1 gm. per liter. With the appearance of albumin in the urine the phenolsulfonephthalein output was decreased, a reduction occurred in the alkali reserve of the blood and in the tension of alveolar air carbon dioxide, and acetone bodies appeared in the urine. Usually during the 2nd day these changes in the urine and blood had reached their maximum and from this point in the experiments the animals showed a return to the normal both in regard to the functional capacity of the kidney, and the restoration of the normal acid-base equilibrium of the blood. Since this series of animals went to the stage of recovery, a study of the experiments permits an investigation of the relation between the elimination of mercury by the kidney and the development of the kidney injury. In Experiment 9, Table IV, on the 1st day of the intoxication a heavy precipitate of mercury was obtained from the urine. The amount of urine formed showed an increase over the

TABLE IV.

Group III. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		*cc*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
7	15	1	621	0	0	0	85	Heavy Tr.	0.012	7.3	8.0	38	Enteritis. No blood.	None.	Good.
		2	297	0	0	5.4114	85	Tr.	0.012	7.4	7.95	34	" improved.	"	Improved.
		4	343	0	0	5.1315	84	"	0.012	7.45	8.05	40	Normal.	"	Appears normal.
		10	497	0	0	11.6625	87	0	0.012	7.55	8.1	45	"	"	Recovery.
8	15	1	655	0.5	0	7.5321	78	Heavy Tr.	0.019	7.4	7.95	38	Severe enteritis. Blood.	Occasional.	Bad.
		2	283	1.0	2.15	12.4210	66	Amount decreased.	0.019	7.4	7.95	38	Improved.	None.	Improved.
		4	450	Tr.	0.2	18.3241	80	0	0.015	7.5	8.1	45	Normal.	"	"
		8	670	0	0	17.6714	82	0	0.015	7.5	8.1	45	"	"	Recovery.
9	15	1	750	0	0	2.5657	85	Heavy Tr.	0.015	7.45	8.0	42	Severe enteritis. No blood.	Occasional.	Good.
		2	580	Tr.	0	6.1885	75	" "	0.026	7.4	7.9	35	Enteritis improved.	"	Improved.
		3	623	"	Tr.	6.2431	83	Amount greatly increased.	0.022	7.5	8.0	40	Improved.	None.	"
		6	220	"	"	11.8957	86	0	0.015	7.45	8.05	40	"	"	"
		10	481	0	0	4.7382	85	0	0.013	7.45	8.05	40	Normal.	"	Normal.
		12	531	0	0	0	85	0	0.013	7.45	8.05	40	"	"	Recovery.

normal, the elimination of phenolsulfonephthalein showed practically no reduction, 86 to 85 per cent, and the reserve alkali of the blood remained unchanged. On the 2nd day of the experiment the elimination of mercury was still heavy, while the formation of urine remained nearly normal. The phenolsulfonephthalein output was reduced only 10 per cent. The alkali reserve of the blood was reduced from 8 to 7.9. On the 3rd day of the experiment the elimination of mercury was greatly increased. There was, however, an increased formation of urine, 623 cc., the output of phenolsulfonephthalein had returned to 83 per cent, the normal output being 86 per cent, and the alkali reserve of the blood had returned to the normal reading. Experiments 7 and 8 also illustrate the lack of relation between the elimination of mercury by the kidney and the decrease in the functional capacity of the organ.

Group IV.

The animals comprising this group developed a moderately severe mercury enteritis. During the enteritis, or following its subsidence, the animals developed an acid intoxication which varied in degree and duration. In this respect this group of animals resembles the experiments of Group III. The animals of Group III, however, gradually returned to the normal and made a complete recovery, while the animals of Group IV, after a period during which there was an attempt to restore the normal acid-base equilibrium, became severely intoxicated and showed a more marked depletion of the alkali reserve than was obtained in the initial acid intoxication. The formation of urine was rapidly reduced. The animals were anuric from 1 to 6 days before death.

A study of Table V, which gives in detail the results obtained in one of the experiments of this group, shows that the secretion of urine was rapidly reduced. Associated with the reduction in the output of urine the animals became albuminuric, and later both glucose and acetone appeared in the urine. The amount of albumin was small, not over 0.9 gm. per liter. The glycosuria rapidly disappeared. The output of acetone bodies in the urine was associated with a reduction in the alkali reserve of the blood but did not show quantitatively

TABLE V.

Group IV. Observations on Acutely Nephropathic Animals.

Experiment No.	Mercuric chloride per kilo.	Day of experiment.	Urine.	Albumin per liter.	Glucose.	Acetone per 100 cc.	Phenolsulfonephthalein.	Mercury in urine.	Blood urea.	pH.	R. pH.	Carbon dioxide tension.	Stools.	Vomitus.	Condition of animal.
	mg.		*cc.*	*gm.*	*per cent*	*mg.*	*per cent*		*per cent*			*mm.*			
10	15	1	870	0.4	Tr.	0	63	Tr.	0.015	7.3	8.05	38	Severe enteritis.	Frequent.	Bad.
		2	344	0.9	0.38	7.9304	47	F. Tr.	0.055	7.2	7.9	36	Enteritis improved.	Occasional.	"
		3	106	0.5	1.1	9.7965	13	Very F. Tr.	0.060	7.25	7.85	33	Improved.	None.	Improved.
		4	277	0.2	Tr.	4.1985	26	" " "	0.068	7.3	7.95	36	Normal.	"	"
		5	178	F. Tr.	"	10.2130	41	0	0.062	7.4	7.95	35	"	"	No change.
		6	232	0	0	14.162	50	0	0.018	7.4	8.0	38	"	"	Improved.
		10	387	0	0	14.7631	51	0	0.029	7.4	7.95	38	"	"	No change.
		12	81	0	0	7.3620	13	0	0.072	7.15	7.75	23	"	"	Bad.
		13	0	0	0	0	0	0	0.092	7.1	7.7	20	None.	"	Died in air-hunger.

a parallel with the decrease in the alkali reserve. As the acid intoxication progressed the eliminaton of acetone bodies was diminished. The decrease in the elimination of phenolsulfonephthalein is not proportionate to the decreased formation of urine. In this group of animals, as in the other groups, there is more nearly a correlation between the degree of acid intoxication and the elimination of the dye than there is between the output of urine and its elimination. Associated with the reduction in the output of phenolsulfonephthalein a retention of blood urea occurs. The stage of improvement in this group of animals was characterized by an increased formation of urine, a decrease and final disappearance of albumin from the urine, an increase in the output of acetone bodies and phenolsulfonephthalein, a decrease in the percentage of blood urea, and a return towards the normal acid-base equilibrium of the blood. None of these conditions reached the point of normality. From the 6th to the 10th day in Experiment 10, the urine was free from albumin, the phenolsulfonephthalein elimination was 48 to 51 per cent, and the reserve alkali 7.9 to 7.95. On the 11th day the terminal acid intoxication commenced. The following day only 81 cc. of urine were formed. The urine was free from albumin. The phenolsulfonephthalein elimination had been reduced from 51 to 13 per cent and the alkali reserve was reduced from 7.95 to 7.75. The animal was anuric the following day and died in air-hunger.

A study of the elimination of mercury by the animals of this group is of special interest. During the entire course of Experiment 10, Table V, only a trace of mercury was found in the urine. After the 4th day of the intoxication the urine was free from mercury. As will be seen by referring to the table, the kidney damage which was associated with the death of the animal did not occur until the 11th day of the experiment, 7 days after the urine had been free from mercury.

Pathology of the Kidney.—The kidneys of this group of animals have been pale and relatively bloodless. Extending from the corticomedullary junction into the cortex are streaks of fatty degeneration. The histological examination has shown the glomeruli shrunken and not infrequently surrounded by an exudate of serum, fibrin, and red cells. The endothelium of the glomerular capillaries has shown an

advanced degeneration. The nuclei have shown fragmentation. The loops of capillaries are fused together. The epithelium of the tubules has shown an advanced necrosis. The structure of the cells is completely lost. The tubules are outlined by a structureless mass of necrotic material. The tubules have frequently been separated by an exudate containing red cells and fibrin. (Fig. 3.)

SUMMARY.

A study of the experiments comprising the first group of animals permits the deduction that these animals succumb to the acute poisoning as a result of the shock which the poison induces through its corrosive action in the stomach and intestine. The animals die before the mercury, acting as such during its elimination by the kidney, can induce an acute nephropathy and before the mercury, by inducing an acid intoxication, can lead to an acute kidney injury.

The remaining animals of the series, Groups II, III, and IV, have withstood the corrosive action of the poison. These animals have shown the same type of delayed intoxication from the poison. The intoxication, however, has varied in time of appearance, duration, and severity.

The animals classified as Group II have developed during the stage of improvement from the gastroenteritis a rapid and severe type of acid intoxication, have become rapidly anuric, and have died either in a state of air-hunger or in convulsions.

The animals of Group III, either during or after their recovery from the gastroenteritis, have developed a mild grade of acid intoxication. During the following days of the experiments the animals succeeded in reestablishing their normal acid-base equilibrium. All the animals of this group recovered.

The animals of Group IV have shown a recovery from the mercury enteritis. Following a period during which there was an attempt on the part of the animals to return to normal, as indicated by an increase in the alkali reserve of the blood and by an increased output of phenolsulfonephthalein and urine, the members of the group developed a delayed acid intoxication, and, like the animals of Group II, became anuric.

The animals of all groups which have died from the delayed intoxication caused by the mercury have shown a severe type of kidney injury which has been characterized by an acute swelling and necrosis of the renal epithelium. All these animals have either gradually or acutely developed a severe type of acid intoxication. There has been a definite association between the development of an acid intoxication and the delayed kidney injury, and, furthermore, the animals which have shown the greatest swelling and necrosis of the renal epithelium have also shown the severest type of intoxication.

CONCLUSIONS.

1. In the acute mercuric chloride intoxications which have been induced in dogs death has been due either to the shock associated with the severe mercury enteritis or to a delayed kidney injury.

2. The injury to the kidney has been constantly associated with the development of an acid intoxication.

3. The delayed kidney injury is not due to the action of the mercury as such during its elimination by this organ.

4. The manner in which mercuric chloride induces an acid intoxication is at present under investigation. The participation of the liver in the intoxication will be considered in this connection.

EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 3, Table II. The glomerulus, *a*, appears normal except for the deeply staining endothelial nuclei. The epithelium of the convoluted tubules, *b*, shows the presence of albuminous granules with little increase in the size of the epithelial cells. The nuclei stain well. The convoluted tubule epithelium at *c* shows an early swelling of the cells. The animal died in collapse associated with the corrosive action of mercuric chloride.

PLATE 15.

FIG. 2. Camera lucida drawing, Zeiss oc. 3, obj. 6. The figure is from the kidney of Experiment 6, Table III. It shows at *a* the histologically well preserved glomerular capillaries. The endothelial nuclei are swollen and stain intensely. At *b* is shown the severe swelling of the convoluted tubule epithelium

which has occluded the lumen of the tubule and increased the size of the tubule. The nuclei of these cells are large and hypochromatic. At *c* are shown tubules that have become completely necrotic. The junctional tubules, *d*, do not show the severe swelling and necrosis which has developed in the more highly specialized tubular epithelium. The animal became anuric from the mercuric chloride intoxication and died in air-hunger.

PLATE 16.

FIG. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of Experiment 10, Table V. It shows at *a* the glomerulus with fused capillary loops containing endothelial nuclei which have undergone fragmentation. The convoluted tubules at *b* have become completely necrotic. The outline of the tubule is formed of a structureless, necrotic mass. At *c* an exudate is shown separating the tubules, which consists of serum, fibrin, and red blood cells. The figure illustrates the changes in the kidney of the animals of Group IV, Table V, which recover from the mercury enteritis and later succumb from the delayed intoxication which is associated with the development of an acid intoxication.

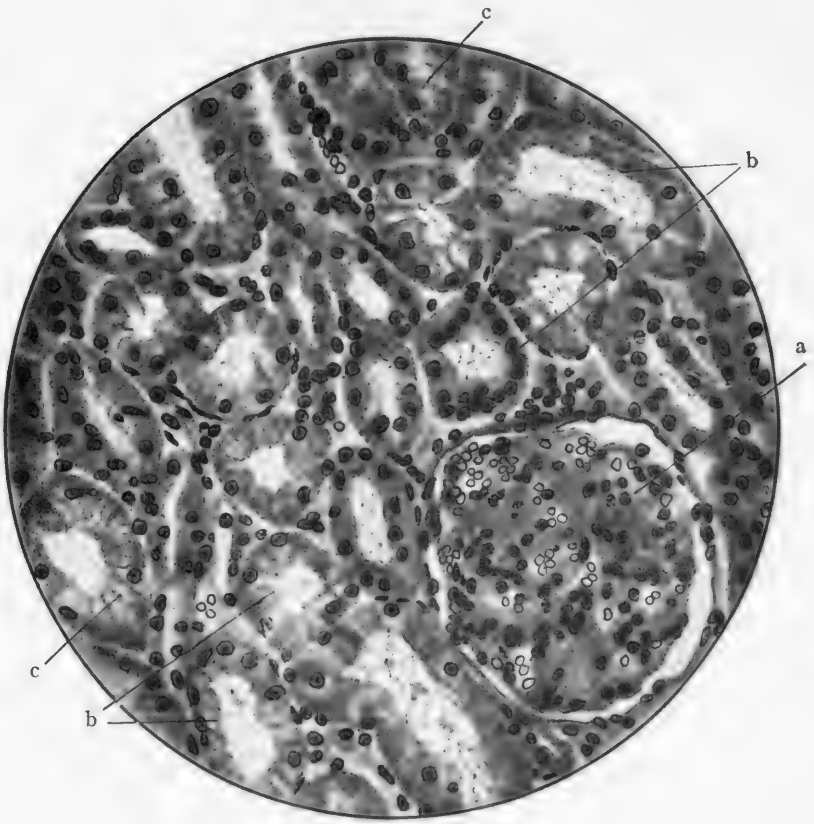

FIG. 1.

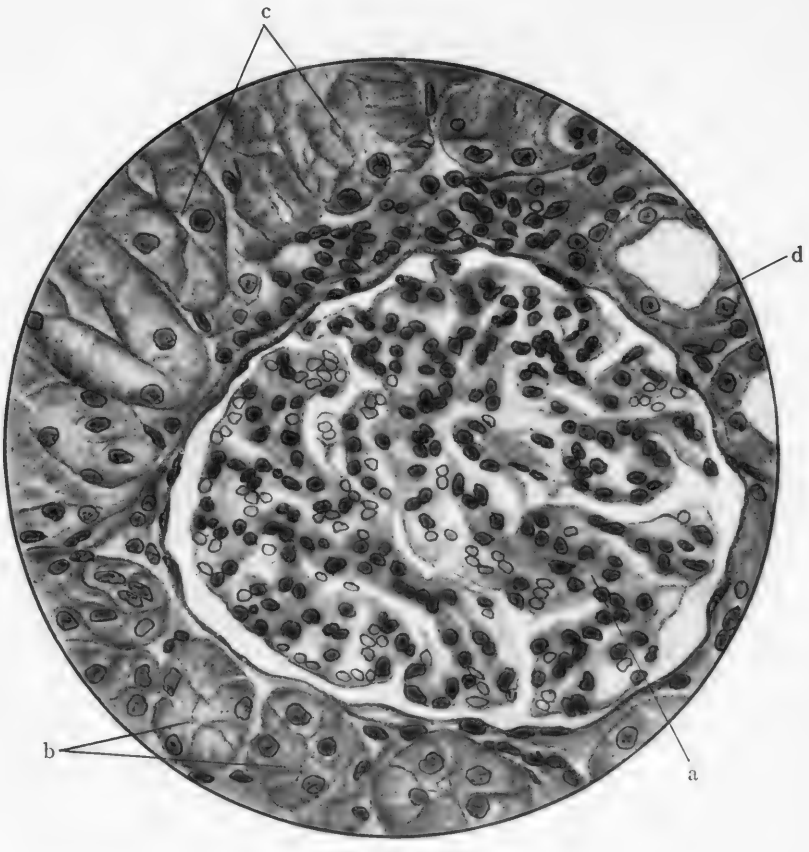

FIG. 2.

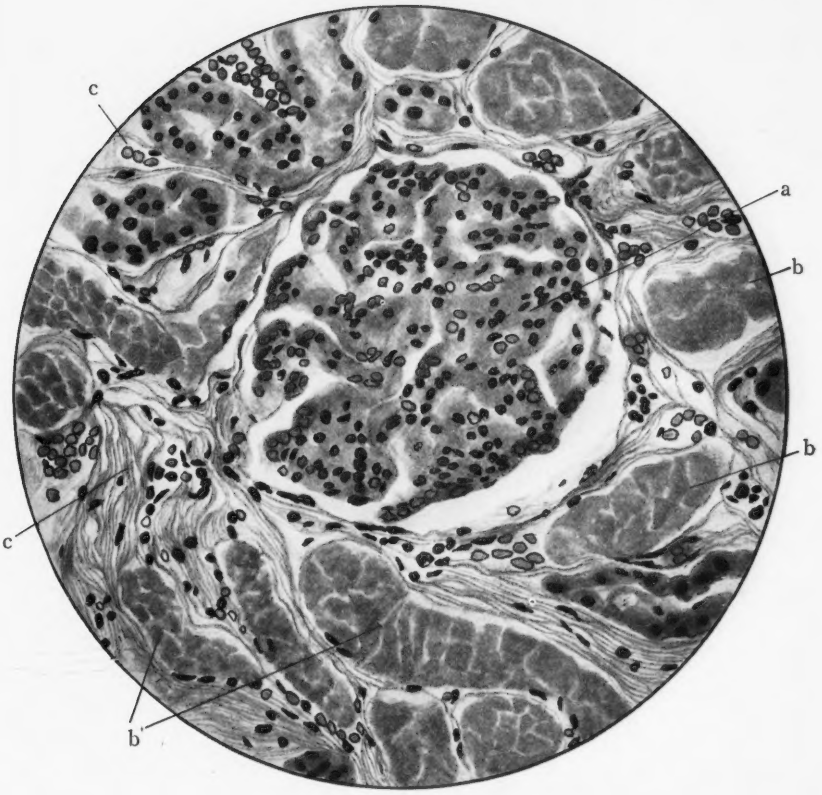

FIG. 3.